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THE UNIVERSITY OF ALBERTA

AMINO ACID METABOLISM
BY
RUMEN PAPILLAE

BY

ⓒ ROY JOHN BOILA

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Amino Acid Metabolism by Rumen Papillae" submitted by Roy John Boila in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Biochemistry.

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ABSTRACT

Rumen papillae, prepared from sections of cattle rumen mucosal lining obtained from a local abattoir, were incubated aerobically with various concentrations and combinations of ammonium chloride, amino acids and organic acids, the latter including propionate, pyruvate, α-ketoglutarate and glyoxylate. Incubation media were analyzed using a gas-liquid chromatographic technique entailing separation of isobutyl-N(O)-heptafluorobutyryl amino acid esters: glutamine was recovered with glutamate and asparagine with aspartate.

Of the amino acids released by rumen papillae, only alanine, glycine, serine, aspartate (plus asparagine) and glutamate (plus glutamine), or ornithine with arginine as a substrate, were released in quantities greater than that measured in incubations without added nitrogen or carbon sources.

Alanine release by rumen papillae was increased in the presence of pyruvate. An increased release of alanine with propionate was only observed with the inclusion of glutamate as a nitrogen source.

Glycine was released by rumen papillae incubated with glyoxylate. However, additions of glycolaldehyde, glyoxal or glycollate, as potential sources of glyoxylate, did not influence release of glycine. Incubation with 1 mM ethanolamine increased glycine release slightly. Oxalate (1 and 10 mM) increased the release of amino acids, but did not influence glycine selectively.

Incubation of rumen papillae with α-ketoglutarate increased release of glutamate plus glutamine. Of the amino acids released by rumen papillae in response to added substrates, serine and aspartate plus asparagine were not quantitatively major products.

Glutamate was an effective amino donor for the production of alanine from pyruvate and of glycine from glyoxylate. Alanine also served as a source of nitrogen for the synthesis of glycine from glyoxylate.

Methionine sulfoximine, reported to be a non-competitive inhibitor of glutamine synthetase (EC 6.3.1.2), was added to incubation media to prevent the synthesis of glutamine. However, it was not possible to demonstrate unequivocally that glutamine was a portion of total glutamate plus glutamine present in incubation media. When rumen papillae were incubated with glutamine plus carbon sources, the glutamine disappeared, but did not influence the release of amino acids. Approximately 3% of the glutamine included in incubation media was recovered as glutamate plus glutamine after the incubation period; glutamate recovery was approximately 55% of that added to incubation media.

Incubations with added serine (0.1 to 2 mM) increased release of glycine markedly by rumen papillae. Only with the inclusion of 1 mM formaldehyde or formate with 1 mM glycine was serine release by rumen papillae increased. An active serine hydroxymethyltransferase (EC 2.1.2.1) in the tissues of rumen papillae was indicated.

With 1 mM arginine as a substrate, there was a release of ornithine by rumen papillae, indicating urea production.

The possible incorporation of rumen fluid ammonia nitrogen into amino acids, and the release of urea from arginine and ammonia nitrogen from glutamine by rumen papillae, or ruminal epithelium are discussed with respect to the transfer of nitrogen between the host animal and the rumen.

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1. INTRODUCTION

Tracer studies involving the use of [^{15}N] ammonium salts and [^{14}C] or [^{15}N] urea infused intraruminally or intravenously have provided quantitative estimates of flows of these compounds in sheep (Pilgrim et al. 1970; Mathison and Milligan 1971; Nolan and Leng 1972; Nolan et al. 1976). Inputs of nitrogen to the rumen include dietary nitrogen, as well as blood urea nitrogen, which enters either via saliva (Somers 1961), or from plasma to rumen fluid across the ruminal epithelium (Houpt and Houpt 1968; Houpt 1970). The outputs of nitrogen from the rumen include microbial nitrogen and non digested dietary nitrogen to the omasum, and ammonia nitrogen (NH_3-N) to the omasum or as a direct flow from rumen fluid to blood plasma across ruminal epithelium (Lewis et al. 1957; Hogan 1961).

Nolan and Leng (1972), after injecting [^{15}N] ammonium chloride intraruminally or [^{15}N] urea intravenously to sheep, reported that only 38% of the urea which entered the digestive tract was degraded to NH_3-N and reentered the blood urea nitrogen pool. Part of this [^{15}N] urea nitrogen contributed to the rumen fluid NH_3-N pool, which in turn was a source of nitrogen for the synthesis of microbial protein. Nolan and Leng (1972) suggested that rumen fluid NH_3-N can be incorporated into nitrogenous compounds, such as amides or non essential amino acids in the intestinal wall or liver which are subsequently incorporated into slowly equilibrating pools of nitrogen, such as body protein. Mathison and Milligan (1971) and Havassy et al. (1974) proposed that the synthesis of glutamate or glutamine may represent an incorporation of rumen fluid NH_3-N into nitrogenous compounds.

The intent of this study was to determine the possibility of amino acid synthesis, the substrates that might be used for synthesis of amino acids and the pathways of synthesis that may occur in ruminal epithelium. To achieve these goals, rumen papillae were incubated with a source of NH_3-N (ammonium chloride), carbon sources and amino acids. Incubation media were subsequently analyzed for amino acids using a gas-liquid chromatographic technique. Alanine, glycine, serine, aspartate, asparagine, glutamate and glutamine were accorded particular emphasis as these amino acids have been suggested to be involved in the transport of carbon and nitrogen between tissues in rats (Aikawa et al. 1973) and sheep (Wolff et al. 1972; Wolff and Bergman 1972a, 1972b).

2. LITERATURE REVIEW

An association between the rumen population of bacteria and protozoa and the host animal allows ruminants to maintain bodily function while consuming diets having low available nitrogen contents (Leng 1973), or to adapt to intakes of large quantities of nitrogen (Clarke et al. 1966). On diets of a low nitrogen content, the total nitrogen available for microbial protein synthesis is supplemented by plasma urea nitrogen recycling to the rumen (Somers 1961; Houpt 1970). Rumen fluid ammonia nitrogen (NH_3-N), produced as a result of degradation of protein or non-protein dietary nitrogen by rumen microorganisms may reach relatively high concentrations. When large quantities of dietary nitrogen were fed daily, Clarke et al. (1966) attributed the loss of total protein nitrogen between the dietary intake of nitrogen and abomasal nitrogen content, to the absorption of NH_3-N from the rumen. The NH_3-N produced from urea, a non protein dietary nitrogen supplement, may predispose the ruminant to a condition of ammonia toxicity (Chalupa 1972). Both non toxic and toxic influences and consequences of rumen fluid NH_3-N are discussed in the following review of the literature.

2.1 Microbial Fermentation in the Rumen

2.1.1 Nitrogen Metabolism by Rumen Microorganisms

Dietary protein is degraded by rumen microorganisms to NH_3-N and volatile fatty acids (Blackburn 1965). Proteolytic activity has been associated with both bacteria and protozoa (Blackburn and Hobson 1960), with the end products of the initial stages of protein degradation being amino acids and polypeptides (Abou Akkada and Blackburn 1963). Amino acids have not been found in rumen fluid to a significant degree (Leibholz 1965, 1969) since they are readily deaminated to NH_3-N and the respective carbon skeletons (Tillman and Sidhu 1969), which in turn are degraded to volatile fatty acids, or used as substrates for the synthesis of amino acids and microbial protein by rumen microorganisms (Walker 1965).

Rumen bacteria have adapted to NH_3-N as a readily available source of nitrogen for growth. Of the 89 freshly isolated strains of rumen bacteria prepared by Bryant and Robinson (1962), 82% grew well on media containing ammonium sulfate and a small amount of cysteine as the main sources of nitrogen. Several bacterial cultures exhibited a requirement for the amino acids of casein hydrolysate.

A limit to the utilization of NH_3-N as a substrate for microbial protein synthesis was found at 50 mg NH_3-N per litre of rumen fluid by Satter and Slyter (1974), as judged from the results of sequential additions of urea as a nitrogen supplement to continuous-culture fermenters charged with rumen contents from a steer fed a purified, an all concentrate or a forage-concentrate (23:77) diet. The growth of bacteria utilizing NH_3-N was not enhanced by increasing rumen fluid NH_3-N beyond 50 mg NH_3-N per litre. Extending these in vitro results to the rumen in vivo indicates that, at higher concentrations of NH_3-N in rumen fluid, nitrogen is lost from the rumen as NH_3-N.

The utilization of NH_3-N for amino acid and protein synthesis by microorganisms in the rumen liquor from sheep is influenced by the chemical form of dietary carbohydrate (Reis and Reid 1959). Addition of glucose, a readily fermentable substrate to rumen liquor in vitro, reduces

NH_3-N accumulation by stimulating the utilization of NH_3-N for the synthesis of microbial protein. The starch of dietary concentrates is more rapidly used as a carbon source for microbial protein synthesis than is cellulose, a highly indigestible carbohydrate found in roughages (Church 1975).

When the supply of dietary nitrogen was less than 16 g nitrogen per day as protein nitrogen fed to sheep (Clarke et al. 1966) or 9 g nitrogen per day as urea nitrogen in a purified diet fed to sheep (Hume et al. 1970), there was a net transfer of blood urea nitrogen into the rumen, either via salivary secretions (Somers 1961) or a direct transfer across ruminal epithelium (Houpt and Houpt 1968; Houpt 1970). The feeding of diets of a high nitrogen content which cause high rumen fluid NH_3-N concentrations (Leibholz 1969; Leibholz and Hartmann 1972) would result in a net absorption of NH_3-N into the portal system of sheep (Lewis et al. 1957). Absorption of NH_3-N directly from the rumen (Hogan 1961) reduces the total nitrogen which flows out of the rumen of animals fed the high nitrogen diets (Clarke et al. 1966; Hume et al. 1970).

2.1.2. Carbohydrate Metabolism by Rumen Microorganisms

The production of the volatile fatty acids, acetate, propionate and butyrate as end products of carbohydrate fermentation by rumen microorganisms, falls into distinct patterns determined by the dietary form of carbohydrate (Rumsey et al. 1970). Dietary carbohydrates such as starch, hemicelluloses and cellulose, serve as the major energy-yielding substrates for rumen microorganisms (Walker 1965). A roughage diet of high cellulose content fed to ruminants is associated with a rumen fluid pH nearer neutrality and a higher molar acetate to propionate ratio than is a concentrate diet of high starch content fed ad libitum. Concentrate diets result in a rapid release of total volatile fatty acids into rumen fluid by rumen microorganisms within 2 hours of feeding (Rumsey et al. 1970).

Volatile fatty acids themselves are substrates for the synthesis of cellular constituents by rumen bacteria (Hoover et al. 1963). Conversion of volatile fatty acids to bacterial protein, nucleic acids and polysaccharides appears to be quite extensive, with acetate serving as the major substrate.

2.1.3. The Output of Nitrogen and Carbon From the Rumen

The main fermentation products including NH_3-N, the volatile fatty acids, and bacterial and protozoal protein are either absorbed directly or flow out of the rumen and may be absorbed further down the gastrointestinal tract. Amino acids of microbial and unfermented dietary protein are absorbed from the small intestine after proteins are hydrolyzed through the action of intestinal proteases and peptidases; NH_3-N and volatile fatty acids are absorbed directly from the omasum, abomasum or small intestine (Armstrong and Hutton 1975). The dietary inputs of nitrogen are changed in the rumen; the sources of nitrogen available to ruminant body tissues become the amino acids from microbial or non digested dietary protein, microbial nucleic acids and NH_3-N (Smith 1975).

2.2 Ammonia Metabolism in Body Tissues

The following sections of this review will pertain to the metabolism of NH_3-N within the tissues of the ruminant. The NH_3-N pool of the ruminant includes NH_3-N absorbed directly from the gastrointestinal tract plus that produced as a result of aerobic amino acid degradation within the tissues of the ruminant animal. The metabolism of volatile fatty acids, in particular propionate, and other carbon containing compounds such as lactate and amino acids will be discussed as needed in relation to the metabolic activities centred about NH_3-N (Reilly and Ford 1971; Wolff and Bergman 1972a, 1972b; Heitmann et al. 1973; Bergman et al. 1974a, Ballard et al. 1976).

2.2.1 Ammonia in Physiological Fluids

Ammonia is found in the body fluids of ruminants, as well as of other animals. In an aqueous solution ammonia is a strong base with a pKa of about 9.02 at 37 C. At a pH of 7.0 to 7.5, it can be calculated, using the Henderson-Hasselbach equation, that more than 98% of the ammonia exists as the ammonium (NH_4^+) ion (Visek 1968; Lund et al. 1970). An excessive quantity of NH_3-N in body tissues is highly toxic to animals (Visek 1968). Therefore, there are mechanisms of maintenance of low circulating levels of NH_3-N in body fluids.

The quantity of ammonia found in the circulatory system depends upon the sampling site. In rats and man, portal vein whole blood has free NH_3-N levels that are higher (0.18 to 0.26 mM) than are found in blood from the peripheral circulation (0.02 to 0.04 mM) (Lund et al. 1970). In sheep, the level of NH_3-N in portal vein blood is dependent upon the rumen fluid concentration of NH_3-N (Lewis et al. 1957); the concentration of NH_3-N in the peripheral circulation (carotid or jugular blood) was no greater than 0.1 to 0.2 mM. As the rumen fluid NH_3-N reached 60-100 mM after repeated doses of ammonium acetate in sheep, Lewis et al. (1957) were able to discern an increased concentration of NH_3-N in the peripheral circulation.

2.2.2 Incorporation of Ammonia Nitrogen into Amino Acids

Enzyme reactions, as listed in Tables 1 and 2, are responsible for (1) the direct uptake of NH_3-N, (2) the direct release of NH_3-N, (3) the transfer of amino nitrogen (aminotransferases) and (4) the synthesis of urea. The amino acids that may be metabolized by way of the reactions shown include glutamate, aspartate, alanine,glycine, serine, glutamine and asparagine, plus the amino acids of the ornithine cycle (Krebs and Henseleit 1932), citrulline, arginine and ornithine. The majority of amino acid metabolism entailing the reactions in Tables 1 and Table 2 is located in liver, kidney and muscle tissues. These reactions are associated with the metabolism of amino acids which may eventually serve as glucogenic substrates (Wolff and Bergman 1972b), as nitrogen-containing compounds stimulating urea synthesis in liver tissue (Chamalaun and Tager 1970; Saheki and Katunuma 1975) or as sources of NH_3-N excreted by the kidney (Pitts 1964).

2.2.2.1 The Synthesis of Glutamate

The incorporation of NH_3-N into the amino acid glutamate is catalyzed by the enzyme glutamate dehydrogenase (Reaction 1, Fig. 1; Sallach and Fahien 1969). Glutamate dehydrogenases from animal sources have been found to function equally effectively with NAD or NADP as the cofactor (Frieden 1968; Sallach and Fahien 1969). The direction of the reaction depends markedly upon the presence of reduced or oxidized nicotinamide nucleotides and NH_3-N (Krebs et al. 1973). At equilibrium glutamate

Table 1. Enzyme reactions responsible for the incorporation and release of ammonia nitrogen in body tissues.

Enzyme	Enzyme Commission Number	Reaction
A. Incorporation of ammonia nitrogen.		
Glutamate dehydrogenase	1.4.1.2 (NAD) 1.4.1.3 (NAD(P)) 1.4.1.4 (NADP)	L-Glutamate + H_2O + NAD(P) → α-Ketoglutarate + NH_3 + Reduced NAD(P)
Glutamine synthetase	6.3.1.2	ATP + L-Glutamate + NH_3 → ADP + Orthophosphate + L-Glutamine
Carbamoyl-phosphate synthetase	2.7.2.5	2ATP + NH_3 + CO_2 + H_2O → 2ADP + Orthophosphate + Carbamoyl-phosphate
Asparagine synthetase	6.3.1.1	ATP + L-Aspartate + NH_3 → AMP + Pyrophosphate + L-Asparagine
B. Release of ammonia nitrogen.		
Glutaminase	3.5.1.2	L-Glutamine + H_2O → L-Glutamate + NH_3
Asparaginase	3.5.1.1	L-Asparagine + H_2O → L-Aspartate + NH_3
ω-Amidase	3.5.1.3	An ω-Amido-dicarboxylic acid + H_2O → A Dicarboxylic acid + NH_3
Adenosine deaminase	3.5.4.4	Adenosine + H_2O → Inosine + NH_3
L-Serine dehydratase	4.2.1.13	L-Serine → Pyruvate + NH_3

Table 2. Enzyme reactions resposible for transferring amino-nitrogen in body tissues and the synthesis of urea in liver tissue. A. Aminotransferases. B. Ornithine cycle enzymes.

Enzyme	Enzyme Commission Number	Reaction
A. Aminotransferases		
Aspartate aminotransferase	2.6.1.1	L-Aspartate + α-Ketoglutarate → Oxaloacetate + L-Glutamate
Alanine aminotransferase	2.6.1.2	L-Alanine + α-Ketoglutarate → Pyruvate + L-Glutamate
Glutamine-keto-acid aminotransferase	2.6.1.15	L-Glutamine + An α-Keto Acid → α-Ketoglutaramate + An Amino Acid
B. Ornithine cycle enzymes		
Ornithine carbamoyltransferase	2.1.3.3	Carbamoyl-phosphate + L-Ornithine → Orthophosphate + L-Citrulline
Argininosuccinate synthetase	6.3.4.5	ATP + L-Citrulline + L-Aspartate → AMP + Pyrophosphate + L-Argininosuccinate
Argininosuccinate lyase	4.3.2.1	L-Argininosuccinate → Fumarate + L-Arginine
Arginase	3.5.3.1	L-Arginine + H_2O → L-Ornithine + Urea

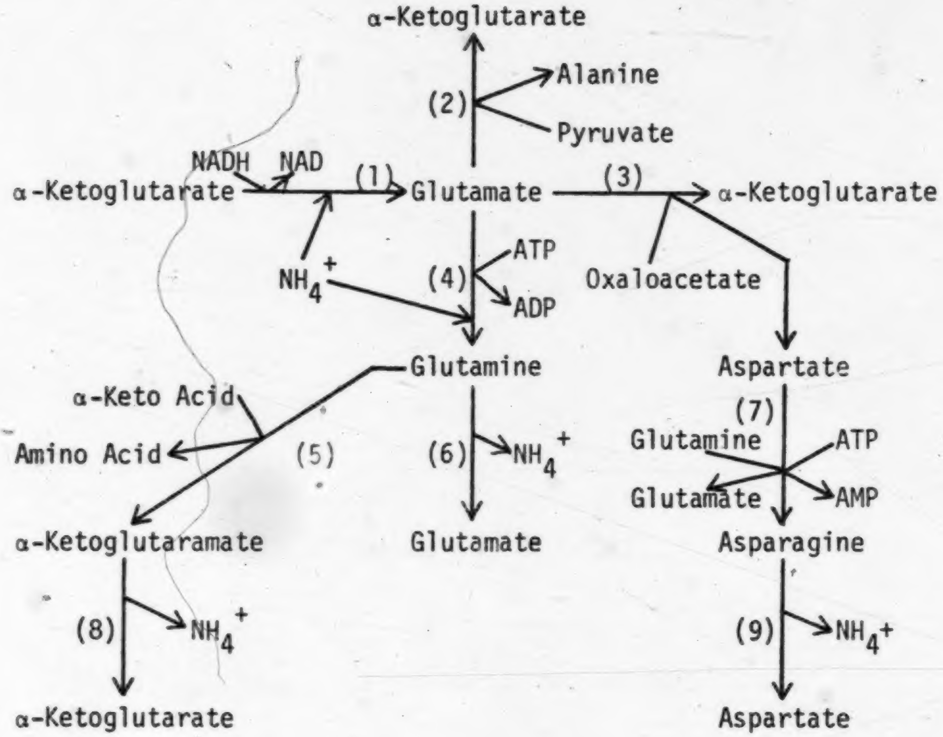

α-Ketoglutarate
Alanine
(2)
Pyruvate
NADH
NAD
α-Ketoglutarate
(1)
Glutamate
(3)
α-Ketoglutarate
ATP
NH_4^+
(4)
Oxaloacetate
ADP
Glutamine
Aspartate
α-Keto Acid
(7)
Glutamine
ATP
Amino Acid
(5)
(6)
NH_4^+
Glutamate
AMP
α-Ketoglutaramate
Glutamate
Asparagine
(8)
NH_4^+
(9)
NH_4^+
α-Ketoglutarate
Aspartate

Fig.1. The synthesis of amino acids with the formation of glutamate as the initial step. (Sallach and Fahien 1969; Greenberg 1969b)

Reaction
(1) Glutamate dehydrogenase
(2) Alanine aminotransferase
(3) Aspartate aminotransferase
(4) Glutamine synthetase
(5) Glutamine-keto-acid aminotransferase
(6) Glutaminase
(7) Asparagine synthetase
(8) ω-Amidase
(9) Asparaginase
Refer to Tables 1 and 2 for the Enzyme Commission number.

formation is favoured (Sallach and Fahien 1969).

2.2.2.2 Amino Donors

The formation of glutamate from α-ketoglutarate and NH_3-N in body tissues is the first step towards the synthesis of many amino acids (Sallach and Fahien 1969; Greenberg 1969b). The amino group of glutamate is transferred to α-keto acids during the synthesis of the amino acids aspartate (Fig. 1), alanine (Fig. 1), serine (Fig. 2) and glycine (Fig. 3). Consequently, these amino acids serve as reservoirs of NH_3-N in body tissues.

With the addition of NH_3-N to glutamate and aspartate, the amides, glutamine and asparagine respectively are formed (Fig. 1). An energy input as adenosine triphosphate is necessary for the synthesis of both amides. Glutamine is more important than asparagine as a source of nitrogen in body tissues because it subsequently participates in the formation of biological compounds such as purines and aminosugars (Greenberg 1969b). The amide nitrogen of glutamine and asparagine may be removed in body tissues in hydrolytic reactions catalyzed by the enzymes glutaminase and asparaginase respectively (Reactions 6 and 9 respectively, Fig. 1, Sallach and Fahien 1969).

Glutamine can serve as an amino donor in a manner similar to glutamate (Cooper and Meister 1973). Glutamine aminotransferase (Table 2; Reaction 5, Fig. 1) has been purified and characterized from rat liver (Cooper and Meister 1972), rat kidney (Cooper and Meister 1974) and from the brain of the bovine (Van Leuven 1974) and of the rat (Van Leuven 1976). The liver enzyme has different properties than the kidney enzyme (Cooper and Meister 1973). Liver glutamine aminotransferase is most active with glutamine, methionine, glyoxylate, pyruvate and α-keto-γ-methiolbutyrate;

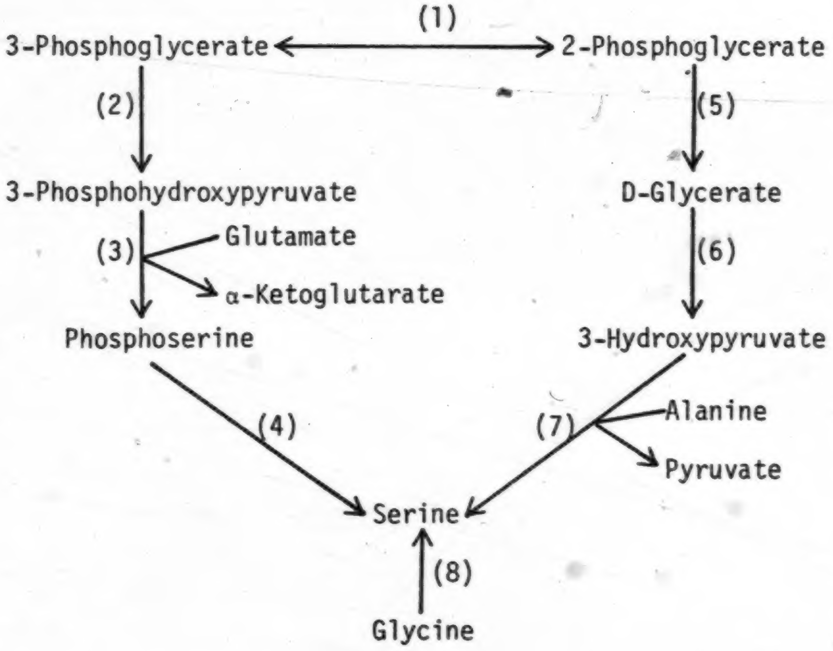

(1)
3-Phosphoglycerate
2-Phosphoglycerate
(2)
(5)
3-Phosphohydroxypyruvate
D-Glycerate
(3)
Glutamate
α-Ketoglutarate
(6)
Phosphoserine
3-Hydroxypyruvate
(4)
(7)
Alanine
Pyruvate
Serine
(8)
Glycine

Fig.2. The biosynthesis of serine. (Greenberg 1969b)

Reaction
(1) Phosphoglyceromutase (EC 2.7.5.3)
(2) Phosphoglycerate dehydrogenase (EC 1.1.1.95)
(3) Phosphoserine aminotransferase (EC 2.6.1.52)
(4) Phosphoserine phosphatase (EC 3.1.3.3)
(5) Glycerate kinase (EC 2.7.1.31)
(6) Glycerate dehydrogenase (EC 1.1.1.29)
(7) Serine-pyruvate aminotransferase (EC 2.6.1.51)
(8) Serine hydroxymethyltransferase (EC 2.1.2.1)

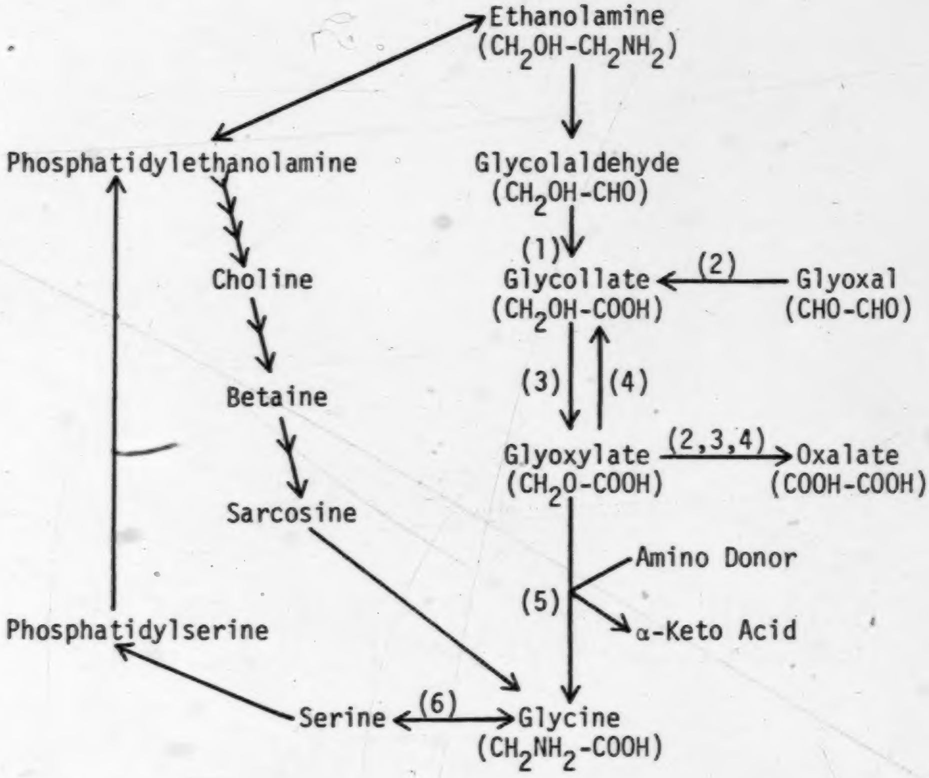

Ethanolamine
($CH_2OH-CH_2NH_2$)
Phosphatidylethanolamine
Glycolaldehyde
($CH_2OH-CHO$)
(1)
Choline
Glycollate
($CH_2OH-COOH$)
(2)
Glyoxal
($CHO-CHO$)
(3)
(4)
Betaine
Glyoxylate
($CH_2O-COOH$)
(2,3,4)
Oxalate
($COOH-COOH$)
Sarcosine
Amino Donor
(5)
α-Keto Acid
Phosphatidylserine
Serine
(6)
Glycine
(CH_2NH_2-COOH)

Fig.3. The biosynthesis of glycine. (Arnstein 1954; Meister 1965; Hagler and Herman 1973)

Reaction
(1) Aldehyde dehydrogenase (EC 1.2.1.3) or Aldehyde oxidase (EC 1.2.3.1)
(2) Xanthine oxidase (EC 1.2.3.2)
(3) Glycollate oxidase (EC 1.1.3.1)
(4) Lactate dehydogenase (EC 1.1.T.27)
(5) Amino acid-glyoxylate aminotransferase
(6) Serine hydroxymethyltransferase (EC 2.1.2.1)

kidney glutamine aminotransferase is most active with glutamine, methionine, phenylalanine, tyrosine and the α-keto acids of these amino acids as substrates, and exhibits relatively little activity towards glyoxylate and pyruvate. Also, it was postulated by Cooper and Meister (1973) that other than the synthesis of alanine and glycine, glutamine aminotransferase may function to convert small amounts of the α-keto acid precursors of methionine, phenylalanine and tyrosine to the respective amino acids. Although the reaction catalyzed by glutamine aminotransferases are reversible, the rapid conversion of α-ketoglutaramate to α-ketoglutarate and ammonia by the enzyme ω-amidase (Reaction 8; Fig. 1) essentially limits glutamine aminotransferase to the formation of amino acids from the precursor α-keto acids (Cooper and Meister 1973).

2.2.2.3 The Synthesis of Glycine and Serine

The biosyntheses of serine (Greenberg 1969b) and glycine (Thompson and Richardson 1966, 1967) are possible through separate pathways, or interconversion of these amino acids (Greenberg 1969a) may be catalyzed by serine hydroxymethyltransferase (EC 2.1.2.1) (Figs. 2 and 3). Serine can be synthesized through a pathway entailing non-phosphorylated intermediates with alanine as the amino donor, or a pathway with phosphorylated intermediates with glutamate as the amino donor (Greenberg 1969b; Fig. 2). Glycine is derived either from serine or a direct transamination of glyoxylate (Fig. 2). Amino donors for the conversion of glyoxylate to glycine included glutamate in human (Thompson and Richardson 1966) or rat (Nakada 1964) liver, glutamine in rat liver, kidney or brain (Cooper and Meister 1973; Van Leuven 1976) and alanine in human liver (Thompson and Richardson 1967).

Compounds examined as sources of glyoxylate in rat liver tissue include ethanolamine, glycolaldehyde, glycollate, glyoxal and oxalate (Weinhouse and Friedman 1951; Kun 1952; Weissbach and Sprinson 1953a, 1953b). Ethanolamine is converted to glyoxylate with glycolaldehyde and glycollate as intermediates (Weissbach and Sprinson 1953b; Fig. 3). Serine is a potential source of ethanolamine through a transformation of phosphatidylserine to phosphatidylethanolamine (Meister 1965; Fig. 3). Glyoxal, an aldehyde, is converted by xanthine oxidase (EC 1.2.3.2) to glycollate (Dixon and Lutwak-Mann 1937), which in turn is oxidized to glyoxylate. Glyoxylate can be converted to oxalate (Hagler and Herman 1973); however, non-reversible formation of glycine may help to prevent excessive formation of oxalate from glyoxylate (Thompson and Richardson 1967).

2.2.2.4 Miscellaneous Reactions

Urea, synthesized through a cyclic series of reactions entailing the amino acids citrulline, arginine and ornithine as intermediates (Krebs and Henseleit 1932; Fig. 4), is a major nitrogenous excretory product of mammals, including ruminants (McIntyre 1970). The immediate nitrogen donors in the cycle of urea synthesis are carbamoyl phosphate and aspartate (Fig. 4). In animal tissues urea is an inert compound; only urease (EC 3.5.1.5), either produced by microbes in the rumen (Rahman and Decker 1966) or in the caecum (Hecker 1971), is able to catalyze the hydrolysis of urea to ammonia and carbon dioxide.

Adenosine deaminase was included in Table 1 because of its involvement in the release of NH_3-N in muscle tissue (Lowenstein 1972). It catalyzes the conversion of adenosine monophosphate to inosine monophosphate

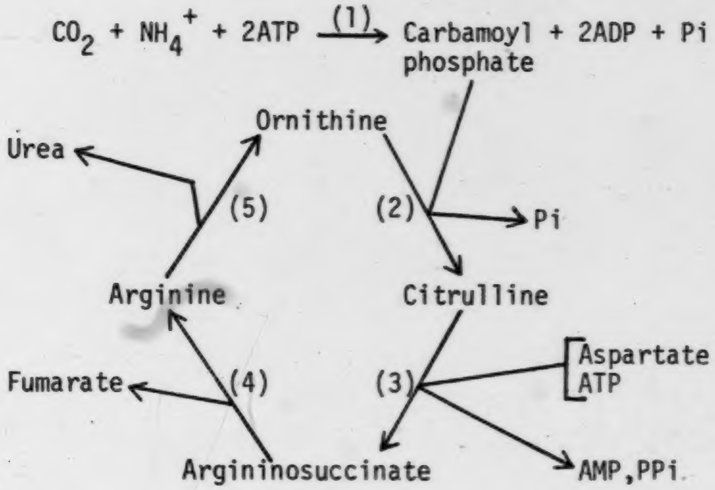

$CO_2 + NH_4^+ + 2ATP \xrightarrow{(1)}$ Carbamoyl phosphate + 2ADP + Pi
Ornithine
Urea
(5)
(2)
Pi
Arginine
Citrulline
Aspartate
ATP
Fumarate
(4)
(3)
Argininosuccinate
AMP,PPi

Fig.4. The ornithine cycle. (Sallach and Fahien 1969)

Reaction
(1) Carbamoyl-phosphate synthetase
(2) Ornithine carbamoyltransferase
(3) Argininosuccinate synthetase
(4) Argininosuccinate lyase
(5) Arginase
Refer to Table 2 for the Enzyme Commission number.

with a release of NH_3-N in response to a heavy work load in muscle.

Enzymes such as serine dehydratase (EC 4.2.1.13) (Table 1), cystine desulphydratase (EC 4.4.1.1) and threonine dehydratase (EC 4.2.1.16) release NH_3-N through a non-oxidative deamination of an amino acid.

2.3 Ammonia Toxicity

Ammonia at low concentration is a normal metabolite in living organisms. However, metabolic evidence of ammonia toxicity may develop when detoxification processes are impaired, or ammonia formation is excessively rapid (Visek, 1968). The ratio of non-ionic ammonia (NH_3) relative to the ammonium (NH_4^+) ion which increases rapidly at pH's above 7.0 to 7.5 (Visek 1968), may contribute to the development of ammonia toxicity (Visek 1972). The flow of non-ionic NH_3 relative to ionic NH_4^+ between tissues was found to increase as the pH of the body fluids increased (Visek 1968).

Ammonia toxicity in sheep was induced by addition of a large dose of urea directly into the rumen (Morris and Payne 1970; Bartley et al. 1976). The most visible aspect of acute ammonic toxicity was the development of tremors of the head in the vertical plane; the magnitude of the tremors increased until a general tetanic spasm indicated death was imminent (Morris and Payne 1970). This urea toxicosis is due to the sudden release of NH_3-N by rumen microorganisms from added urea. High concentrations of rumen fluid NH_3-N may exist without producing toxicity if the ration is readily fermentable and the rumen fluid remains below pH 7.4 (Bartley et al. 1976).

Intermittent consumption of diets containing urea, and subsequent rapid release of the urea nitrogen as NH_3-N by rumen microorganisms, may result in a temporary overload of detoxifying mechanisms for NH_3-N that could lead to a condition of ammonia toxicity. Although the potential of ammonia toxicity is always present in ruminants, the absorption of NH_3-N from the rumen is limited by the acid environment of rumen contents, where the ammonium ion form of NH_3-N predominates (Hogan 1961). Any NH_3-N that does become absorbed is fixed into non-toxic forms of nitrogen such as urea (Goldsworthy et al. 1968) and amino acids (Wolff et al. 1972) in liver tissues. Ruminants are able to adapt to levels of NH_3-N in the body, levels which may be indicative of a subacute stage of ammonia toxicity.

2.4 Interorganal Relationships of Amino Acid Metabolism

The release to plasma of an amino acid as an end product of metabolism by one organ, and uptake and metabolism of this amino acid as a substrate by another organ will be designated an interorganal relationship of amino acid metabolism. The amino acids noted to be transported between brain, muscle, kidney, liver and the non-hepatic splanchnic bed as carriers of nitrogen and carbon are alanine, glycine, glutamate, glutamine, aspartate and asparagine, plus the amino acids of the ornithine cycle, including arginine, ornithine and citrulline. Metabolic activities in which interorganal transport of amino acids is involved include the output of amino acids by muscle tissue (Aikawa et al. 1973; Ballard et al. 1976), gluconeogenesis in liver (Wolff and Bergman 1972b) and kidney (Kaufman and Bergman 1974), utilization of amino acids as an energy source by the non-hepatic splanchnic bed (Windmueller and Spaeth 1974, 1975, 1976), the synthesis of urea by liver tissue (Chamalaun and Tager 1970; Saheki and Katunuma 1975), and the production and excretion by the kidney of NH_3-N derived from glutamine (Pitts 1964; Pitts et al.1965).

The amino acids most abundant as carriers of nitrogen and carbon in the vascular system of fasted (Aikawa et al. 1973) or fed rats (Yamamoto et al. 1974) are alanine and glutamine (Fig. 5). Alanine fluxes are a part of the glucose-alanine cycle (Felig 1973; Fig. 6). The glucose-alanine cycle is similar to the Cori cycle (Cori 1931), with alanine replacing lactate. Glutamine is released by muscle tissues (Marliss et al. 1971; Ballard et al. 1976), and utilized as an energy source by the non-hepatic splanchnic bed (Windmueller and Spaeth 1974), or as a source of NH_3-N by the kidney (Pitts et al. 1965). The nitrogen for alanine and glutamine production by muscle tissues is derived from the degradation of amino acids (Garber et al. 1976).

The representation in Fig. 5 for fasted rats, as reported by Aikawa et al. (1973), was a modification of an earlier scheme presented by Ishikawa et al. (1972) where only glutamine and alanine fluxes were noted. In fed rats (Yamamoto et al.1974), glutamine is utilized rather than released by the liver. The metabolic activities of serine and glycine were only definable for certain tissues (Aikawa et al.1973); serine is an end product of amino acid metabolism in the kidney (Fig. 5) and is believed to be metabolized to glycine by tissues other than kidney. Alanine and glutamine, rather than glycine or serine are important as carriers of nitrogen and carbon in the circulatory system of the rat (Aikawa et al. 1973; Yamamoto et al. 1974).

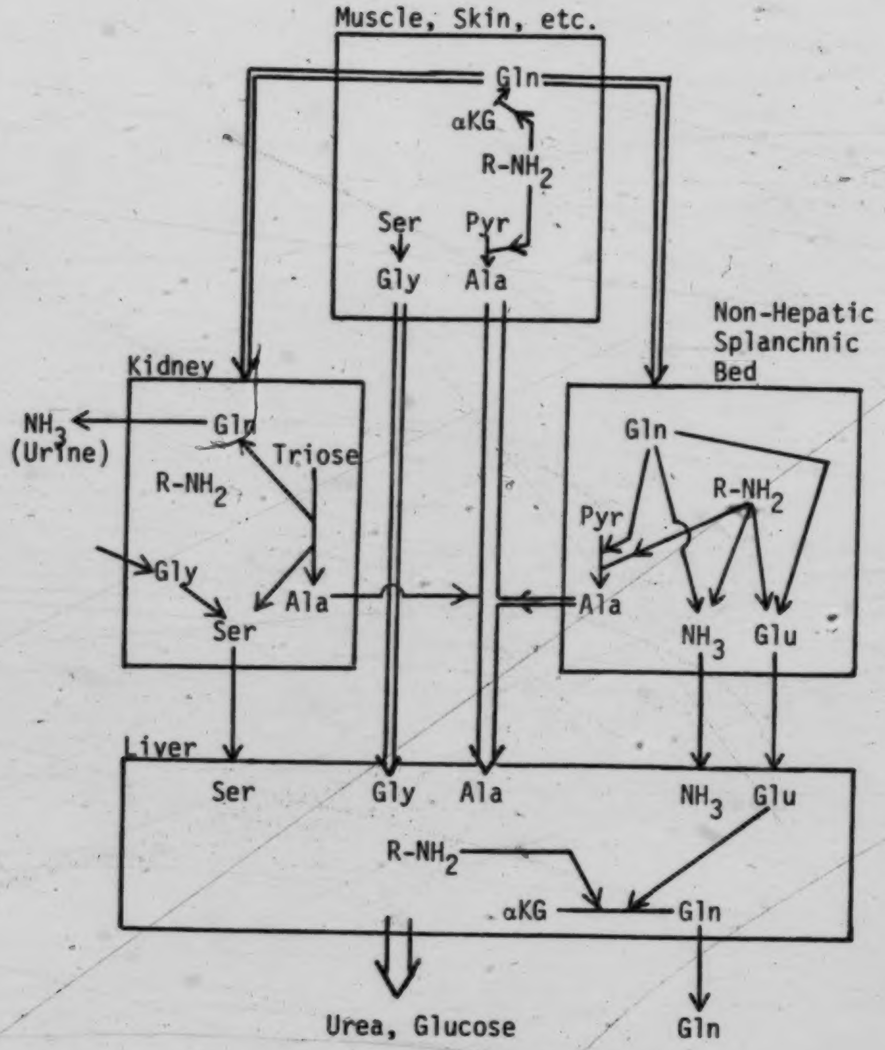

Muscle, Skin, etc.
Gln
αKG
R-NH2
Ser
Pyr
Gly
Ala
Kidney
NH3
(Urine)
Gln
Triose
R-NH2
Gly
Ser
Ala
Non-Hepatic
Splanchnic
Bed
Gln
R-NH2
Pyr
Ala
NH3
Glu
Liver
Ser
Gly
Ala
NH3
Glu
R-NH2
αKG
Gln
Urea, Glucose
Gln

Fig.5. The interorganal relationships of amino acid metabolism for fasted rats. (Aikawa et al. 1973)

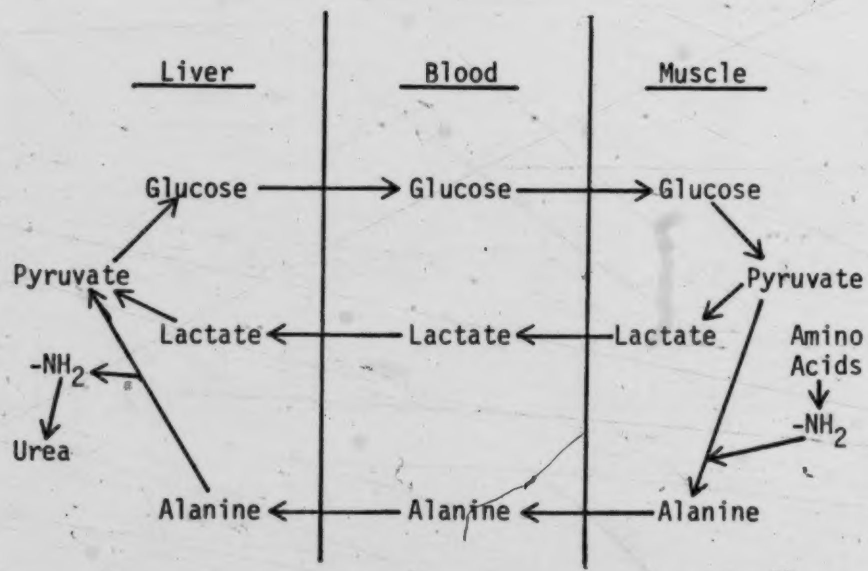

Liver
Blood
Muscle
Glucose
Glucose
Glucose
Pyruvate
Pyruvate
Lactate
Lactate
Lactate
Amino Acids
$-NH_2$
$-NH_2$
Urea
Alanine
Alanine
Alanine

Fig.6 The interrelationship between the Cori (glucose-lactate) cycle and the glucose-alanine cycle. (Cori 1931; Felig 1973)

2.4.1 Urea Synthesis by Liver Tissue

The production of urea by the reactions of the ornithine cycle (Krebs and Henseleit 1932) is localized exclusively in liver tissue (Fig. 4). However, several of the ornithine cycle enzymes have been found singly in kidney (Sallach and Fahien 1969), red blood cells (Barey and Harmeyer 1973; Owczarczyk and Barej 1975; Springell 1975) and rumen epithelium (Martincic and Krvavica 1964; Krvavica et al. 1964; Ide 1969). When there is an increased intake of dietary protein, there is an increase in activity of the enzymes of the ornithine cycle in rat liver as a result of either an increase in total enzyme available or an increased activity of existent enzymes (Schimke 1962; Das and Waterlow 1974; Saheki et al. 1975).

Nitrogen source specificity for urea synthesis has been investigated in both liver perfusion systems and liver slice studies. Ammonia nitrogen added as ammonium salts to perfusion media, is taken up by liver tissue and almost quantitatively converted to urea (Chamalaun and Tager 1970). Of the amino acids in the circulatory system, only glutamine, aspartate, alanine or ornithine in liver perfusion media stimulate urea synthesis (Chamalaun and Tager 1970; Saheki and Katunuma 1975). The inability of glutamate to augment urea synthesis in perfusion studies (Goldsworthy et al. 1968) may have been due to a permeability barrier restricting the entry of glutamate through the liver cell membrane (Hems et al. 1968). In liver homogenates in which cell membranes were disrupted, glutamate readily served as a nitrogen donor for urea synthesis (Hems et al. 1968). Ammonia nitrogen and the amino acids, alanine and glutamine were the major sources of urea nitrogen in liver perfusion studies (Chamalaun and Tager 1970).

2.4.2 Metabolism of Amino Acids by the Kidney; the Excretion of Ammonia Nitrogen

In monogastrics (rat, dog and man) as compared to sheep (Bergman et al. 1974a), glutamine is the major precursor of the NH_3-N excreted by the kidney of an animal in an acidotic condition. For dogs in an acute or chronic acidosis, 50 to 75% of the total NH_3-N excreted by the kidney is derived from glutamine (Pitts 1964; Pitts et al. 1965). In fed, pregnant sheep, glutamine is added to the renal vein by the kidney, while in fasted pregnant sheep, glutamine is removed. Bergman et al. (1974a) suggested that the release of glutamine by sheep kidney is associated with the presence of an active glutamine synthetase. An active kidney glutamine synthetase is found for animals such as guinea pig, rabbit and sheep which produced a neutral or alkaline urine as compared to dogs, whose kidneys contain no glutamine synthetase activity and produce an acidic urine.

Plasma amino acids taken up or released by the kidney of dogs and sheep, other than glutamine, are glycine, serine, alanine and aspartate. Glycine is extracted from plasma to a small degree by the kidney of acidotic dogs, while serine and alanine are added to the plasma (Shalhoub et al.1963). In the fed or fasted sheep (Bergman et al. 1974a) glycine and serine are always added to renal venous blood by the kidney; alanine and aspartate are always removed by the sheep kidney.

A significant uptake of citrulline was nearly balanced by a net output of arginine by the kidney of fed or fasted sheep (Bergman et al. 1974a). There was a release to the plasma of arginine synthesized from citrulline by the kidney (Bergman et al. 1974a) and an uptake of plasma arginine by the liver (Wolff et al. 1972) with the subsequent release of urea from arginine through the action of liver arginase. Plasma arginine may serve as a major nitrogen carrier between the kidney and the liver of sheep. Urea produced in the liver would be excreted by the kidney, or recycled to the rumen (Houpt 1970) or caecum (Houpt 1963).

2.4.3 Metabolism of Amino Acids by the Non-Hepatic Splanchnic Bed

The amino acids glutamine or glutamate (Windmueller and Spaeth 1974, 1975) and asparagine, aspartate or arginine (Windmueller and Spaeth 1976) were added as [U-^{14}C] labelled substrates to isolated preparations of rat intestine. Glutamine, glutamate or aspartate, whether absorbed from the lumen of the small intestine or taken up from plasma, were metabolized by intestinal tissue to carbon dioxide (up to 60% of added ^{14}C) and lactate (up to 15% of added ^{14}C) as major end products of carbon metabolism. Asparagine was not metabolized by the rat intestinal tissues. The nitrogen of glutamine was approximately accounted for by the nitrogen of the citrulline (34% of glutamine nitrogen), alanine (33%), ammonia (23%) and proline (10%) present in the perfusate after an incubation (Windmueller and Spaeth 1974). About 33% of added arginine was hydrolyzed to ornithine and urea which were released into the blood (Windmueller and Spaeth 1976). Extensive metabolism of glutamine, glutamate, or aspartate by the small intestinal tissue, a part of the non-hepatic splanchnic bed, may indicate that these amino acids are used to meet significant portions of the energy requirement of the small intestine (Windmueller and Spaeth 1976).

2.4.4 Interorganal Amino Acid Movements in Ruminants

Studies of interorganal amino acid flows have been undertaken primarily in fed (Yamamoto et al. 1974) and fasted (Aikawa et al. 1973) rats, and fed dogs (Elwyn et al. 1968). The general principles from these studies also apply for sheep (Wolff et al. 1972; Wolff and Bergman 1972a, 1972b Bergman et al. 1974a; Ballard et al. 1976) and most likely ruminants in general. In studies of the metabolism of amino acids by the liver and portal-drained viscera of sheep (Wolff et al. 1972), the contribution of amino acids and NH_3-N by the rumen to the rest of the portal-drained viscera was not established. The presence of a rumen might change the magnitude or direction of interorganal amino acid fluxes in ruminants, when compared to the basic pattern of fluxes reported to be present in rats (Fig. 5).

The rumen has an influence upon the metabolic relationship between gluconeogenesis and interorganal amino acid fluxes. The almost complete conversion of dietary carbohydrate to volatile fatty acids by rumen microorganisms forces the glucose requirement of the ruminant to be met by gluconeogenesis in the liver and kidney (Bergman et al. 1974b). Alanine, glutamine (glutamate), glycine and serine, as interorganal carriers of nitrogen and carbon, contribute carbon to the glucose turnover in sheep (Wolff and Bergman 1972b; Heitmann et al. 1973). The synthesis of amino acids such as glutamate in the liver (Wolff et al. 1972) or alanine and glutamine in muscle (Ballard et al. 1976) of sheep, would not only fix NH_3-N into non-toxic forms, but also temporarily carry carbon, some of which would subsequently be used as a glucogenic source.

2.5 Interactions of Ruminal Epithelium with the Rumen Environment

The aerobic epithelial lining of the rumen is exposed to abrasive rumen contents and the products of anaerobic microbial fermentation. Histologically this lining was designated as a parakeratotic, psoriatic, stratified squamous epithelium (Lavker et al. 1969), with the epithelium forming papillae over the surface of the rumen. Papillae are numerous and very densely packed, particularly in the ventral sac of the rumen (Church 1975; Weigand et al. 1975). The metabolic capabilities of ruminal epithelium include pathways for the metabolism of volatile fatty acids (Pennington 1952) and the ability to synthesize amino acids (McLaren et al. 1961; Whanger and Church 1970). Fluxes of nitrogen as NH_3-N (Hogan 1961), urea (Houpt 1970) and amino acids (Leibholz 1971a, 1971b) have been recorded between rumen fluid and the tissues of rumen mucosa in contact with this fluid. Mineral exchanges between rumen fluid and plasma across ruminal epithelium are also of note (Keynes and Harrison 1970).

2.5.1 Metabolism of Volatile Fatty Acids by Ruminal Epithelium

The volatile fatty acids, acetate, propionate and the normal and iso-forms of butyrate and valerate are absorbed from rumen fluid and metabolized by the ruminal epithelium (Weigand et al. 1975). Rumen papillae (used in most studies to represent the epithelium) are capable of converting propionate to lactate and pyruvate (Pennington 1952, 1954; Weekes 1972, 1974), and butyrate to the ketone bodies (β-hydroxybutyrate and acetoacetate in vitro (Pennington 1952; Hird and Weideman 1964; Bush and Milligan 1971). Acetate was metabolized to ketone bodies to a limited extent (Pennington 1952; Stevens and Stettler 1966). Very little isobutyrate was metabolized by rumen papillae in vitro (Annison and Pennington 1954; Weigand et al. 1975); n-valerate was metabolized to lactate via propionate, and β-hydroxybutyrate via acetate (Weigand et al. 1975); isovalerate was metabolized to acetoacetate (Weigand et al. 1975).

In contrast to the conclusive in vitro conversion by ruminal epithelium of propionate to lactate and carbon dioxide (Pennington and Sutherland 1956b), Weigand et al. (1972) found that lactate produced in vivo by the ruminal epithelium represented only 4.9% (range 2.5 to 9.1%) of absorbed [2-^{14}C] propionate. This in turn was a marked contrast to the 70% conversion of [1-, 2-, 3- or U-^{14}C] propionate to lactate estimated by Leng et al. (1967). The difference in percent propionate converted to lactate in vivo may be due to the fact that Leng et al. (1967) failed to provide direct proof of where the lactate was produced when jugular blood was analyzed, while Weigand et al. (1972) analyzed portal vein blood to identify whether [^{14}C] propionate absorbed from the rumen, or glucose synthesized from [^{14}C] propionate by the liver was the source of the [^{14}C] lactate. The average figure of 4.9% quoted by Weigand et al. (1972) was a liberal estimate of the conversion of propionate to lactate by ruminal epithelium in vivo; circulating plasma glucose synthesized from labelled propionate was converted to lactate by gastrointestinal tissues and was included in the estimate (Weigand et al. 1972; Weekes and Webster 1974).

2.5.2 Nitrogen Fluxes as Ammonia Nitrogen, Urea and Amino Acids

2.5.2.1 Ammonia Nitrogen

Ammonia nitrogen is freely diffusable across ruminal epithelium from rumen fluid to plasma (Lewis et al. 1957) or into peritoneal fluid

(Chalmers et al. 1971). The total NH_3-N available for absorption is limited by the concentration of NH_3-N in rumen fluid, and the pH of this rumen fluid. The proportion of NH_3 relative to NH_4^+ is determined by the pH of the rumen fluid (Hogan 1961), where at pH's of less than 7.0, 98% or more of the NH_3-N is present as the NH_4^+ ion (Visek 1968). Mooney and O'Donovan (1970) stated that the transport of ammonia was rapid, but passive across the rumen wall in vitro; the rumen wall membranes are more permeable to NH_3 than NH_4^+.

2.5.2.2 Urea

Plasma urea was found to be translocated into the rumen as urea (Houpt and Houpt 1968) or as NH_3-N liberated from this urea by the action of bacterial urease associated with rumen epithelium (Houpt 1970). Ammonia nitrogen, as NH_3, penetrates cell membranes much more rapidly than does urea (Houpt 1970; Mooney and O'Donovan 1970). Bacterial urease, which permeates the cornified layers of rumen epithelium (Rahman and Decker 1966; Chalupa et al. 1970) would act upon urea that diffuses from plasma. This was suggested to be a mechanism for facilitation of diffusion of urea (Houpt 1970) and it was considered that the resultant NH_3-N would diffuse to the blood or into rumen fluid.

In contrast to the facilitated mechanism for urea nitrogen transfer from plasma to rumen fluid hypothesized by Houpt (1970), Thorlacius et al. (1971) reported that an active transport mechanism may exist for urea in ruminal epithelium. Thorlacius et al. (1971) were able to increase the transfer of urea up to fourfold into a temporarily isolated, washed ventral sac of the rumen of a conscious cow by applying an atmosphere of 100% carbon dioxide to the ruminal epithelium. An increase of up to fourfold would not have been possible by simple diffusion of urea. With the insufflation of carbon dioxide at atmospheric pressure to the rumen under various conditions, Sellers et al. (1964) were able to increase blood flow to the rumen. The increased blood flow alone was not able to account for the urea transfer reported by Thorlacius et al. (1971). The carbon dioxide, as suggested by Thorlacius et al. (1971), may trigger the production of an unknown intermediary which alters the permeability of ruminal epithelium to urea. A mediated transport mechanism for urea may exist in ruminal epithelium.

2.5.2.3 Amino Acids

A flux of amino acids across ruminal epithelium from mucosa to serosa has been documented (Demaux et al. 1961; Cook et al. 1965; Leibholz 1971a, 1971b). This movement of amino acids across the epithelium was considered to be a diffusion process rather than a mediated transport (Leibholz 1971b). However, the very low concentrations of amino acids found in rumen fluid would not be conducive to extensive amino acid movements across ruminal epithelium and into plasma, where the concentrations of free amino acids are manifold those present in rumen fluid (Leibholz 1965).

2.5.3 Amino Acid Metabolism by Ruminal Epithelium

Enzymes of amino acid metabolism that have been found in ruminal epithelium include glutamate dehydrogenase and aminotransferases that will act upon ornithine (EC 2.6.1.13), tyrosine (EC 2.6.1.5) aspartate, alanine, tryptophan (EC 2.6.1) and phenylalanine (EC 2.6.1) (Whanger and Church 1970; Chalupa et al. 1970). Several enzymes which participate in urea synthesis in liver tissue are present in ruminal epithelium; these include carbamoylphosphate synthetase in cattle (Salem et al. 1973a), ornithine carbomyltransferase in cattle (Krvavica et al. 1964; Whanger and Church 1970) and goat (Ide 1969), and arginase in cattle (Martincic and Krvavica 1964; Whanger and Church 1970).

The presence of a complete ornithine cycle in rumen muscosa is highly unlikely. Chalupa et al. (1970) reported that the activities of the ornithine cycle enzymes were very low and quite variable in sheep rumen mucosa; any activity of ornithine cycle enzymes was attributed to a contamination of the mucosa by rumen bacteria. Aliev and Kosarov (1967) and Kosharov et al. (1969) have established that when [^{15}N] ammonium chloride was added to a rumen pouch of sheep, labelled urea soon entered the blood leaving the rumen pouch. Rather than a synthesis of urea by reactions of the ornithine cycle, arginase, present in rumen epithelium (Martincic and Krvavica 1964), may account for the release of urea from arginine available from plasma.

Evidence for the formation of glutamate from α-ketoglutarate by ruminal epithelium was attained with in vitro studies of rumen mucosa homogenates (McLaren et al. 1961) and of intact rumen papillae (Mathison 1972). The ability to synthesize glutamine was less conclusively established, but was indicated by the presence of the enzyme glutamine synthetase (Chalupa et al. 1970; Salem et al. 1973a). Extensive fluxes of nitrogen between rumen fluid and blood plasma across ruminal epithelium have been attributed to the formation or degradation of glutamate or glutamine by ruminal epithelium (Hoshino et al. 1966; Mathison and Milligan 1971; Havassy et al. 1974, Nolan 1975; Nolan et al. 1976).

Since the enzymes for the synthesis of alanine, aspartate, glutamate and glutamine (Whanger and Church 1974, Salem et al. 1973a) are present in ruminal epithelium, and since the synthesis of glycine from glyoxylate (Mathison 1972) and glutamate from α-ketoglutarate (McLaren et al. 1961) by in vitro preparations of rumen papillae have been reported, one may anticipate that ruminal epithelium would be able to synthesize alanine glycine, glutamate, aspartate and glutamine. For the synthesis of amino acids by rumen papillae, rumen fluid NH_3-N would represent a readily available source of nitrogen, while organic acids such as α-ketoglutarate, pyruvate and propionate could be carbon sources. These amino acids, in turn, would contribute to the interorganal flux of nitrogen and carbon as plasma free amino acids in ruminants.

3. MATERIALS AND METHODS

3.1 Transport of Tissue

Cattle rumen tissue was transported in a Krebs-Ringer Phosphate (Umbreit et al.1972) minus calcium (KRPC) buffer pH 7.6 from a local abattoir (Gainers Limited) to the University of Alberta. Two covered 1 litre styrofoam cannisters of KRPC buffer and one covered 1 litre cannister of 0.9% (wt/vol) NaCl, in a small styrofoam chest were taken to the abattoir. The KRPC buffer and 0.9% NaCl, which had been prepared (sources of chemicals, Table 3) and stored at 0 C in closed 2 litre glass containers, were equilibrated overnight in a water bath at 41 C. Before adding the KRPC to two styrofoam cannisters, the KRPC was aerated with O_2/CO_2 (95/5; vol/vol) for approximately 5 min. It had been determined that, allowing for travel time, the temperature of the KRPC and 0.9% NaCl at the abattoir would be 39 ± 1 C.

Rumen tissue was obtained directly from the plant line with an interval after death of the animal estimated to be about 20 min. A section of rumen tissue (ruminal epithelium with associated muscle layers) was excised from a highly papillated area of the ventral sac of the rumen. A company employee had previously washed the rumen contents out of the intact rumen with cold tap water. The excised tissue was immediately washed successively in warm 0.9% NaCl and warm KRPC, and transported in the third styrofoam cannister containing warm KRPC to the University. In the laboratory, the muscle layers were separated from the epithelium, and the epithelium was placed in KRPC at 39 C aerated with O_2/CO_2. Rumen papillae were clipped from the epithelium with scissors and kept in KRPC at room temperature (22 C) until weighed and added to the incubation flasks, or prepared for dry matter analysis.

3.2 Incubations

Incubation of six series of treatments (Series I to VI) were conducted in 25 ml Erlenmeyer flasks fitted with red rubber serum bottle stoppers (Fisher Scientific Company; Fair Lawn, N.J., U.S.A.). A hole in the stopper (2 mm diameter) was fitted with a short length of solid glass rod as a plug. The hole was used to flush the incubation flasks with O_2/CO_2. Capped Erlenmeyer flasks, each containing 4 ml of prepared incubation medium, were refrigerated until required for incubation with rumen papillae. Prepared incubation media were refrigerated a maximum of 7 days; the average time interval was 4 to 5 days. The flasks were equilibrated in a water bath shaker (Eberbach Corporation; Ann Arbor, Mich., U.S.A.; 60 oscillations per min) at 39 C during the 1 h period while rumen tissue was being obtained from the abattoir.

Approximately 400 mg of blotted rumen papillae were weighed and added to each flask. The flasks were flushed with O_2/CO_2 for 1 to 2 min and were incubated for 3 h at 39 C in the water bath shaker (60 oscillations per min). Immediately upon completion of the 3 h incubation, 100 μl of cycloleucine (2.5 μmoles per ml aqueous 0.1 N HCl), as an internal standard, were added to each incubation flask, and the flask was swirled gently. The total incubation medium with added internal standard was transferred to a 13 x 100 mm culture tube (Canlab; Edmonton, Alta., Canada) containing 0.4 ml of 8 N acetic acid (Harris et al. 1961). This capped culture tube was kept on ice until further preparation was undertaken.

The percent dry matter of rumen papillae was determined in quadruplicate analyses on each sample of fresh rumen papillae used for incubation preparations. Approximately 400 mg of blotted rumen papillae were weighed into 4 dr Rigo plasticlear vials (Johns Scientific; Toronto, Ont., Canada). The papillae were freeze dried, weighed and percent dry matter was calculated (Appendix Table 1).

Amino acids were collected from the incubation medium with added internal standard using a short ion exchange procedure (Appendix A). The freeze dried residues of isolated amino acids were, after appropriate preparation, subjected to gas-liquid chromatography as the isobutyl-N(O)-heptafluorobutyryl amino acid esters (Appendix B).

3.3 Preparation of Incubation Media

The chemicals used in buffers and as substrates are listed in Table 3. Incubation media were prepared for each treatment of Series I to VI with a complete Krebs-Ringer Bicarbonate (KRB) buffer (Umbreit et al. 1972) in 100 ml quantities. The proper weights or volumes of substrates were added to a separate 250 ml beaker for each treatment. Chemicals that were obtained in liquid form were prepared in a concentrated solution of KRB and appropriate volumes were added to the 250 ml beaker. To these substrates were added 50 ml of KRB. This mixture was neutralized to pH 7.0 with 0.1 N HCl or 0.1 N NaOH (10 N NaOH for drastic pH differences) and brought up to volume in a 100 ml volumetric flask with KRB that had been adjusted to pH 7.0 separately. Incubation media were stored in closed plastic bottles at 0 C until measured out into incubation flasks.

Table 3. Sources for buffer chemicals and substrates.

Company	Chemicals
Aldrich Chemical Company (Milwaukee,Wis.,U.S.A.)	1-aminocyclopentanecarboxylic acid (cycloleucine).
J.T.Baker Chemical Company (Phillipsburg,N.J.,U.S.A.)	citric acid, formaldehyde(40%), magnesium sulphate, potassium chloride, potassium phosphate (monobasic), sodium bicarbonate, sodium chloride, sodium lactate (60% syrup).
Fisher Scientific Company (Fair Lawn,N.J.,U.S.A.)	glucose (dextrose), glycollic acid, monoethanolamine, oxalic acid (dihydrate), sodium acetate, sodium formate, sodium phosphate (dibasic anhydrous), succinic acid.
K and K Laboratories Inc. (Plainview,N.Y.,U.S.A.)	Sodium-n-butyrate.
Mallinkrodt Chemical Works (St. Louis,Mo.,U.S.A.)	ammonium chloride (AR).
Mann Research Laboratories (New York,N.Y.,U.S.A.)	L-serine.
Matheson, Coleman and Bell (East Rutherford,N.J.U.S.A.)	malonic acid.
Merck and Company Inc. (Montreal,Que.,Canada)	glycine.
Sigma Chemical Company (St. Louis,Mo.,U.S.A.)	L-alanine, L-arginine-HCl, L-glutamic acid, L-glutamine, glycolaldehyde, glyoxal, glyoxylic acid (monohydrate), hydroxylamine-HCl, hydroxy-L-proline, α-ketoglutaric acid, methionine sulfoximine, malic acid, L-ornithine-HCl, sodium propionate, sodium pyruvate.

3.4 Treatments of Series I to Series VI

Incubations were undertaken in six series (Series 1 to VI). Each series was conducted with three separate fresh tissue preparations on three different days. On each day of incubations of fresh rumen papillae, a blank incubation (no added nitrogen or carbon sources, 0 + 0) for Series I to VI and an incubation containing ammonia nitrogen (NH_3-N) as NH_4Cl and no carbon sources (NH_3-N + 0) for Series I to III were prepared in duplicate. These duplicates were the first and last treatments prepared on each day (Note Table 4 as an example). The average of the duplicates for the 0 + 0 and NH_3-N + 0 incubations were reported. Preparation of approximately 20 incubation flasks on each day took 45 to 60 min. Within the repeatability of the methods of analysis, the different intervals of time in KRPC did not appear to have an influence upon the release of amino acids by rumen papillae for the 0 + 0 or NH_3-N + 0 incubations. The incubations were performed between November 1975 and April 1976.

Rumen papillae were subjected to the treatments in Tables 4 to 9 for Series I to VI respectively. For Series I, 3N NH_4OH was used to elute amino acids from the cation exchange resin (Appendix A); for Series II to VI, 1 N NH_4OH was used. Otherwise, the general procedure was followed.

Series I (Table 4) served as an introduction to Series II to VI. In Series I, 1 mM NH_3-N as NH_4Cl was added to incubations with 10 mM concentrations of organic acids which are intermediates and end products of rumen microbial fermentation (Hungate 1966), or are those that may be present in the body tissues of ruminants (Prior et al.1972).

The effectiveness of glutamate and glutamine as amino donors in the presence of glyoxylate or pyruvate for the synthesis of glycine and alanine

Table 4. Treatments of Series I.

Treatment Number	Nitrogen Source 1mM	Carbon Source 10mM
1	0	0
2	NH_4Cl	0
3	NH_4Cl	Propionate
4	NH_4Cl	Lactate
5	NH_4Cl	Pyruvate
6	NH_4Cl	Glyoxylate
7	NH_4Cl	α-Ketoglutarate
8	NH_4Cl	Succinate
9	NH_4Cl	Malate
10	0	0
11	NH_4Cl	0

respectively was examined in Series II (Table 5). In addition, treatments with α-ketoglutarate plus NH_3-N, and glutamate plus NH_3-N were included for study of glutamate and glutamine synthesis respectively. The effect of propionate as a carbon source was examined with α-ketoglutarate plus NH_3-N, glutamate or glutamine as nitrogen sources (treatments 12, 13 and 14, Table 5). One treatment was prepared with alanine as the nitrogen source and glyoxylate as a carbon source for the synthesis of glycine (treatment 3, Table 5).

The objectives of the treatments of Series III(Table 6) were to examine: two-carbon compounds as substrates for the synthesis of glycine via glyoxylate (treatments 3 to 7 inclusive); glycine synthesis in the presence of 5, 10 and 20 mM glyoxylate (treatments 7, 8 and 9); and amino acid synthesis with citrate, α-ketoglutarate and glucose as carbon sources (treatments 10, 11 and 12). In addition, the effects of incubating NH_4Cl at concentrations greater than 1 mM (treatments 15, 16 and 17) were examined.

In Series IV (Table 7), the treatments were designed to demonstrate the interconversion of serine and glycine by a reaction like that catalyzed by serine hydroxymethyltransferase (EC 2.1.2.1).

The treatments of Series V (Table 8) were similar to those of Series II. Methionine sulfoximine (MS), a non-competitive inhibitor of glutamine synthetase (Meister 1969), was added to incubation media. The effect of added MS (1 mM) was studied in three combinations of nitrogen and carbon sources, with four incubations in each combination. The combinations of nitrogen and carbon sources were: no added glutamate or organic acids (treatments 1 to 4, Table 8); glutamate plus pyruvate (treatments 5 to 8, Table 8); and glutamate plus glyoxylate (treatments 9 to 12,,Table 8).

Table 5. Treatments of Series II.

Treatment Number	Nitrogen Source 1 mM	Carbon Source 10 mM
1	0	0
2	NH_4Cl	0
3	Alanine	Glyoxylate
4	NH_4Cl	Glyoxylate + α-ketoglutarate[1]
5	Glutamate	Glyoxylate
6	Glutamate + NH_4Cl	Glyoxylate
7	Glutamine	Glyoxylate
8	NH_4Cl	Pyruvate + α-ketoglutarate[1]
9	Glutamate	Pyruvate
10	Glutamate + NH_4Cl	Pyruvate
11	Glutamine	Pyruvate
12	NH_4Cl	Propionate + α-ketoglutarate[1]
13	Glutamate	Propionate
14	Glutamine	Propionate
15	0	0
16	NH_4Cl	0

1. The concentration of α-ketoglutarate was 1 mM.

Table 6. Treatments of Series III.

Treatment Number	Nitrogen Source[1]	Carbon Source[1]
1	0	0
2	NH_4Cl	0
3	NH_4Cl	Glycolaldehyde
4	NH_4Cl	Glyoxal[2]
5	NH_4Cl	Glycollate
6	NH_4Cl	Oxalate
7	NH_4Cl	Glyoxylate
8	NH_4Cl	Glyoxylate (5mM)
9	NH_4Cl	Glyoxylate (20mM)
10	NH_4Cl	Citrate
11	NH_4Cl	α-Ketoglutarate
12	NH_4Cl	Glucose (Dextrose)
13	0	0
14	NH_4Cl	0
15	NH_4Cl (3mM)	0
16	NH_4Cl (5mM)	0
17	NH_4Cl (10mM)	0

1. Except where otherwise noted, the concentration of the added nitrogen source was 1 mM and the concentration of added carbon source was 10 mM.
2. Glyoxal was prepared as the 10 mM monomeric equivalent.

Table 7. Treatments of Series IV.

Treatment Number	Amino Acid	Concentration mM
1	0	---
2	Serine	0.1
3	Serine	0.5
4	Serine	1.0
5	Serine	2.0
6	Glycine	0.25
7	Glycine	0.5
8	Glycine	1.0
9	Glycine	2.0
10	Glycine	4.0
11	0	---

Table 8. Treatments of Series V.

Treatment Number	Nitrogen Source[1]	Carbon Source[2]	Other Additions[3]
1	0	0	---
2	0	0	MS
3	NH_4Cl	0	---
4	NH_4Cl	0	MS
5	Glutamate	Pyruvate	---
6	Glutamate	Pyruvate	MS
7	Glutamate + NH_4Cl	Pyruvate	---
8	Glutamate + NH_4Cl	Pyruvate	MS
9	Glutamate	Glyoxylate	---
10	Glutamate	Glyoxylate	MS
11	Glutamate + NH_4Cl	Glyoxylate	---
12	Glutamate + NH_4Cl	Glyoxylate	MS
13	Alanine	0	---
14	Alanine	Glyoxylate	---
15	Alanine	Glyoxylate	HYD
16	0	0	HYD
17	Arginine	0	---
18	0	Hydroxyproline	---

1. The concentration of each nitrogen source was 1 mM.
2. The concentration of each carbon source except hydroxyproline was 10 mM. Hydroxyproline was 1 mM.
3. Other additions included 1 mM methionine sulfoximine (MS) and 2 mM hydroxylamine (HYD).

The four incubations within each combination of nitrogen and carbon sources were: no added NH_3-N; no added NH_3-N plus added MS; added NH_3-N; and added NH_3-N plus added MS.

The activity of alanine as an amino donor in the presence of glyoxylate was further examined in Series V (treatments 13 to 16, Table 8). Hydroxylamine, reported to be an inhibitor of L-alanine-glyoxylate aminotransferase (EC 2.6.1.44) (Thompson and Richardson 1967) was included in treatments 15 and 16 (Table 8).

Two amino acids, arginine and hydroxyproline (treatments 17 and 18 respectively of Table 8) were incubated with rumen papillae. Arginine was added as a substrate for the enzyme arginase which was previously reported to be present in rumen mucosa (Martincic and Krvavica 1964). The release of ornithine by rumen papillae was used as an indicator of urea production. Hydroxyproline, which can be converted to glyoxylate in beef or rat liver (Adams 1971) was included in incubations to determine if it served as a source of glyoxylate in rumen papillae.

In Series VI (Table 9), treatments were prepared to elaborate on results from Series I to IV. These treatments were:

(1) inclusion of 1 mM oxalate and 1 mM malonate as metabolic inhibitors (treatments 3 and 4, Table 9). This allowed a comparison of the 1 mM oxalate treatment with the earlier 10 mM oxalate treatment in Series III (treatment 6, Table 6);

(2) additions of glycine and one-carbon compounds (formaldehyde and formate) to examine effects on serine release by rumen papillae (treatments 5, 6 and 7, Table 9). These incubations were prepared to examine further the interconversions of glycine and serine noted with the

Table 9. Treatments of Series VI

Treatment[1] Number	Nitrogen[2] Source	Carbon Source
1	0	0
2	NH_4Cl	0
3	NH_4Cl	Oxalate (1mM)
4	NH_4Cl	Malonate (1mM)
5	Glycine	0
6	Glycine	Formaldehyde (1mM)
7	Glycine	Formate (1mM)
8	NH_4Cl	Acetate (10mM)
9	NH_4Cl	Propionate (10mM)
10	NH_4Cl	Butyrate (10mM)
11	0	Ethanolamine (1mM)
12	Glutamate + NH_4Cl	Propionate (10mM)
13	Glutamate + NH_4Cl	Propionate (20mM)
14	0	0
15	0	0
16	Glutamate + NH_4Cl	Propionate (10mM)
17	0	0
18	Glutamate + NH_4Cl	Propionate (10mM)

1. Treatments 1 to 14 were incubated for 3 h, treatments 15 and 16 for 2 h, and treatments 17 and 18 for 1 h.
2. The concentration of all added nitrogen sources to all treatments was 1 mM.

treatments of Series IV (Table 7).

(3) incubations of the volatile fatty acids, acetic, propionic and butyric (treatments 8, 9 and 10, Table 9) were included to determine the influence of the ketogenic substrates acetate and butyrate (Hird and Weideman 1964; Stevens and Stettler 1966) and a glucogenic substrate propionate (Bergman et al. 1966; Weekes 1974), upon amino acid release by rumen papillae;

(4) ethanolamine incubated with rumen papillae (treatment 11, Table 9) as a possible source of glyoxylate (Meister 1965), in addition to the incubation of two-carbon compounds in Series III (Table 5);

(5) incubations of 10 and 20 mM propionate to examine the effect of an increased propionate concentration upon amino acid metabolism by rumen papillae, with a combination of 1 mM glutamate and 1 mM NH_3-N as nitrogen sources (treatments 12 and 13, Table 9); and

(6) analysis of incubation media from the blank incubation (treatments 1, 14, 15 and 17, Table 9) and the 1 mM glutamate plus 1 mM NH_3-N plus 10 mM propionate treatment (treatments 12, 17 and 19, Table 9) after 1, 2 and 3 h of incubation to determine the time course of amino acid changes.

Incubation media containing oxalate (treatment 6, Table 6 and treatment 3, Table 9) were prepared without added calcium in KRB. No calcium oxalate precipitate was visible after incubations were completed.

3.5 Calculations

The concentrations of amino acids (AA) in the incubation media were obtained using Relative Molar Ratio (RMR) calculations (Gehrke et al. 1968) with an internal standard (IS) of cycloleucine where:

$$\mathrm{RMR} = \frac{A_{AA}}{A_{IS}}$$

A_{AA} = integrated area of amino acid

A_{IS} = integrated area of internal standard

For a standard (STD) mixture (amino acids at 2.5 μmoles per ml aqueous 0.1 N HCl), RMR_{STD} were calculated against cycloleucine as an internal standard (2.5 μmoles per ml aqueous 0.1 N HCl). Amino acids were obtained in kit form (Kit LAA-21; Sigma Chemical Company; St. Louis, Mo., U.S.A.) or as noted in Table 3. Similarly, for the incubation media samples (SMP), RMR_{SMP} were calculated.

The μmoles of each AA per incubation were:

$$\frac{(\mathrm{RMR}_{SMP})}{(\mathrm{RMR}_{STD})}\ (0.25\ \mu\text{moles})$$

The volume of internal standard added to each incubation flask was 100 μl; this volume was equivalent to 0.25 μmoles of cycloleucine.

The μmoles of AA released per gram dry rumen papillae per incubation period were calculated as:

$$\frac{\mu\text{moles of each AA released per incubation flask}}{\text{grams dry weight of rumen papillae per incubation flask}}$$

3.6 Presentation of the Data

Three forms of presentation of the data were adopted:

1. For the amino acids, alanine, glycine, serine, aspartate plus asparagine and glutamate plus glutamine, micromoles of each individual amino acid released into the incubation medium by rumen papillae upon 3 h of

incubation were calculated. These were expressed as μmoles per gram dry rumen papillae per incubation period.

2. For each Series, the averages (μmoles per g per incubation period) for amino acids released by rumen papillae to the incubation medium when substrates were added, were calculated as a percent of the blank incubation. These percent of blank incubation values became a representation of the numerical data in the accompanying figures.

3. Production of each amino acid was calculated as a percent of total production, the total being a sum of μmoles of amino acids (alanine, glycine, serine, aspartate plus asparagine and glutamate plus glutamine) released per gram dry rumen papillae per incubation period. This served to indicate whether one amino acid was synthesized preferentially over another when rumen papillae were incubated with substrates.

3.7 Statistics

Standard errors (Snedecor and Cochran 1967) were calculated for each set of incubations (n = 3) within a Series.

4. RESULTS

4.1 Amino Acid Analysis

The gas-liquid chromatographic (GLC) method of analysis of amino acids (Appendix B) did not separate the pairs including glutamate and glutamine, aspartate and asparagine or ornithine and citrulline. During ester formation, glutamine and asparagine were converted to the esters of glutamate and aspartate respectively, while the esters of citrulline and ornithine had the same retention characteristics when chromatographed. Since it was not possible to separate glutamine from glutamate, or asparagine from aspartate by GLC analysis (Appendix B), these amino acid pairs were reported as glutamate plus glutamine (Glx) and aspartate plus asparagine (Asx).

In a preliminary study of amino acid release by rumen papillae, a thin-layer chromatographic (TLC) procedure involving cellulose plates developed in a solvent of phenol-water (75/25; wt/wt) according to the method of Von Arx and Neher (1963) was used in an attempt to separate glutamine from glutamate, asparagine from aspartate and citrulline from ornithine. Rumen papillae were incubated with no added substrates, with 1 mM NH_3-N, and with 1 mM NH_3-N plus pyruvate, lactate or α-ketoglutarate. It was not possible to use TLC to separate these pairs of amino acids, since glycine was found to co-chromatograph with asparagine, alanine with glutamine and norleucine, the internal standard with ornithine.

4.2 The Treatments of Series I to Series VI

Amino acids other than alanine, glycine, serine, aspartate plus asparagine and glutamate plus glutamine appeared in the incubation media but did not respond to treatments imposed in this study. The recovery of these amino acids, whose calculated RMR did not differ among tissue preparations, was approximately 0.05 to 0.10 μmoles for valine, threonine, isoleucine and proline, and 0.15 μmoles for leucine, phenylalanine, lysine and tyrosine per incubation flask; traces of methionine and arginine were also present. Only with arginine added to incubation medium (Series V) was release of ornithine by rumen papillae noted.

Except in a few treatments where amino acids were added to incubation media as nitrogen sources, the release of total reported amino acids by rumen papillae was between 14 and 37 μmoles per gram dry rumen papillae per incubation period.

4.3 Series I. Ammonia Nitrogen Plus Organic Acids

Incubation in 1 mM NH_3-N (NH_3-N + O) did not change the pattern of amino acid release by rumen papillae when compared to the blank incubation (Fig. 7).

With lactate as a carbon source, glutamate plus glutamine was 65% of the blank incubation; the other amino acids were 87 to 102% of the blank incubation (Fig. 7). Pyruvate increased alanine production to 136% and serine production to 170% of the blank (Fig. 8). With propionate in the incubation medium, alanine release by rumen papillae was decreased to 86% of the blank, and serine release was increased to 321% of the blank (Fig. 8). Expressed as a percent of total amino acids measured, alanine release in the presence of propionate was 46.6%, as compared to 37.1% for the blank (Table 10). Of the carbon substrates in Series I, propionate

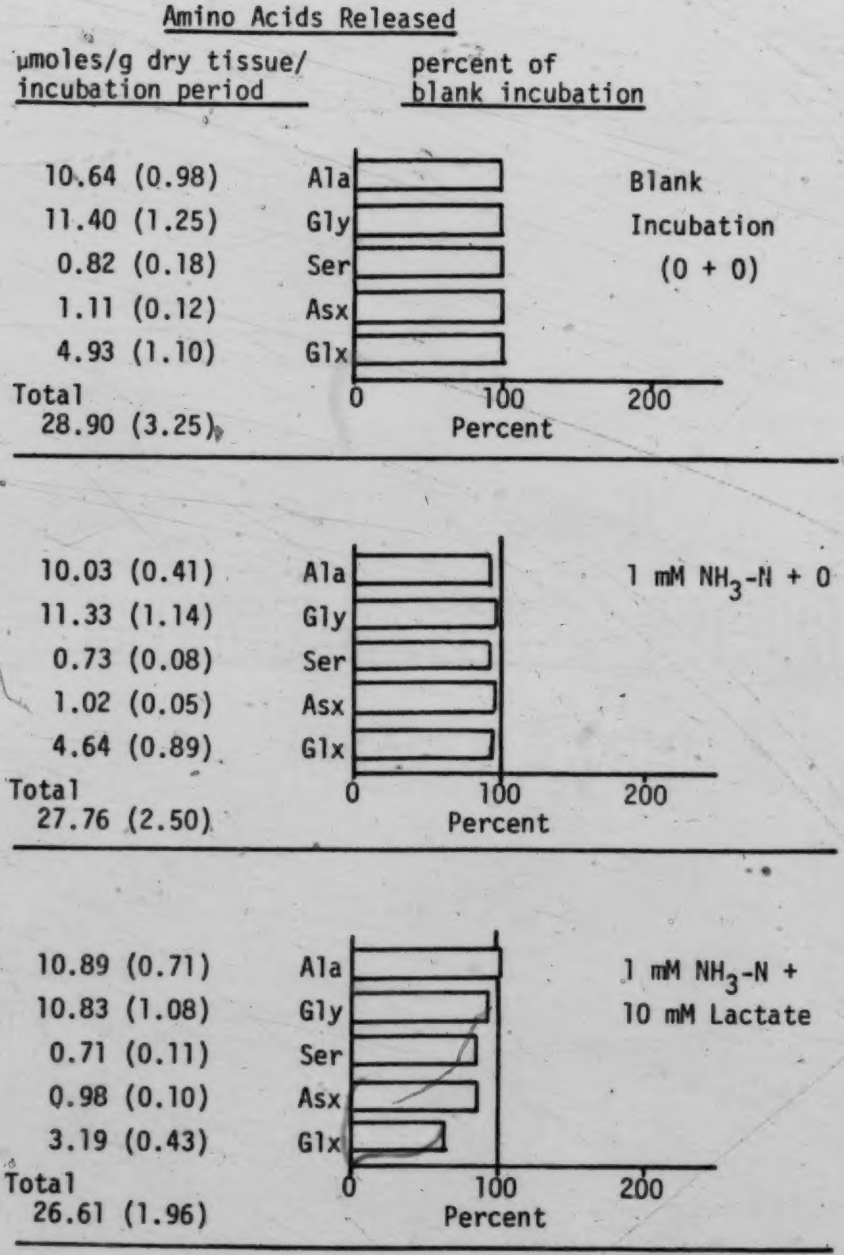


Fig.7. Series I. Amino acid release by rumen papillae incubated with ammonia nitrogen plus lactate.(Mean followed by standard error in parentheses)

Amino Acids Released

	µmoles/g dry tissue/ incubation period
Ala	14.49 (1.91)
Gly	9.86 (1.31)
Ser	1.39 (0.11)
Asx	0.68 (0.10)
Glx	2.44 (0.54)
Total	28.85 (3.68)

	µmoles/g dry tissue/ incubation period
Ala	9.20 (1.40)
Gly	4.83 (0.95)
Ser	2.63 (0.16)
Asx	0.63 (0.10)
Glx	2.42 (0.44)
Total	19.71 (2.84)

	µmoles/g dry tissue/ incubation period
Ala	5.06 (0.61)
Gly	23.64 (3.43)
Ser	2.38 (0.26)
Asx	1.03 (0.19)
Glx	4.74 (1.29)
Total	36,85 (5.19)

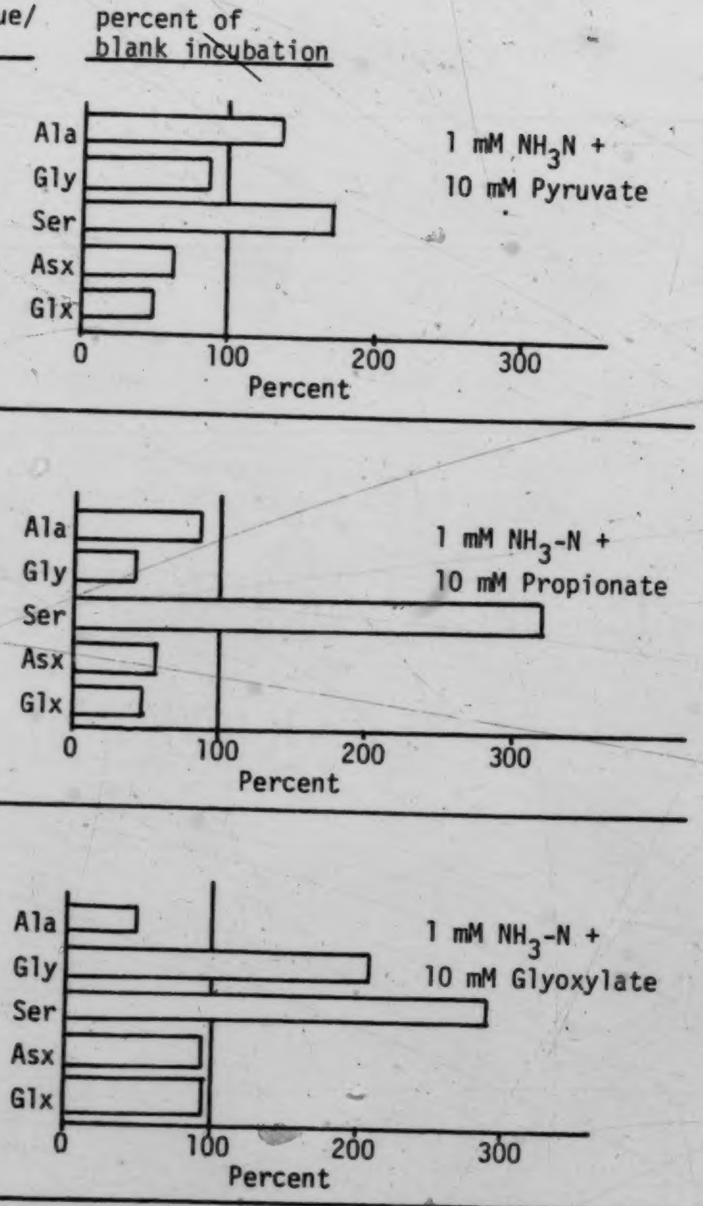


Fig.8. Series I. Amino acid release by rumen papillae incubated with ammonia nitrogen plus pyruvate, propionate or glyoxylate. (Blank incubation Fig.7; mean followed by standard error in parentheses)

Table 10. Series I. Amino acids calculated as a percent of the total reported amino acids released by rumen papillae incubated with ammonia nitrogen plus organic acids.

Treatment	Amino Acid				
	Ala	Gly	Ser	Asx	Glx
Blank Incubation	37.1 (1.2)[1]	39.6 (0.9)	2.8 (0.4)	3.9 (0.1)	16.1 (2.0)
NH_3-N + O [2]	36.5 (2.0)	40.8 (0.6)	2.6 (0.1)	3.7 (0.2)	16.4 (1.8)
NH_3-N + Lactate [3]	41.0 (1.7)	40.6 (2.5)	2.6 (0.2)	3.7 (0.3)	12.0 (1.3)
NH_3-N + Pyruvate	50.2 (1.2)	34.1 (0.4)	4.9 (0.3)	2.4 (0.1)	8.5 (1.4)
NH_3-N + Propionate	46.6 (1.2)	24.2 (1.2)	13.8 (1.8)	3.2 (0.2)	12.2 (1.1)
NH_3-N + Glyoxylate	13.8 (0.5)	64.1 (2.3)	6.5 (0.3)	2.8 (0.2)	12.8 (2.4)
NH_3-N + α-Ketoglutarate	16.3 (4.5)	32.0 (0.9)	3.3 (0.2)	1.5 (0.2)	46.5 (5.3)
NH_3-N + Succinate	40.1 (1.5)	32.0 (0.4)	7.2 (0.3)	5.3 (0.6)	15.3 (1.8)
NH_3-N + Malate	37.7 (1.3)	40.2 (0.5)	3.1 (0.5)	7.9 (1.5)	11.2 (1.3)

1. Mean (n=3) followed by standard error in parentheses.
2. The concentration of NH_3-N for all treatments was 1 mM.
3. The concentration of organic acid in all treatments was 10 mM.

resulted in the lowest total for amino acids (19.71 μmoles per g tissue per incubation period; Fig. 8) present in the medium at the end of incubation.

During incubation with 10 mM glyoxylate, glycine release by rumen papillae was 207% of the blank incubation, while serine release was 290% of the blank incubation (Fig. 8). Calculated as a percent of total, glycine represented 64.1% of the total reported amino acids in the incubation medium (Table 10). This glycine increase (Fig. 8) was the largest absolute response for any amino acid in Series I.

The addition of α-ketoglutarate to the medium increased glutamate to 295% of the blank and serine to 126% of the blank (Fig. 9). Succinate increased serine to 220% of the blank (Fig. 9). With added malate, aspartate plus asparagine was increased to 185% of the blank (Fig. 9).

The incubation of rumen papillae with 1 mM NH_3-N plus 10 mM pyruvate, propionate, glyoxylate, α-ketoglutarate or malate in Series I resulted in the release of less amino acids by rumen papillae with each of these treatments in the range of 40 to 60% of the blank incubation. These amino acids included: aspartate plus asparagine and glutamate plus glutamine with pyruvate (Fig. 8) present in incubation media; glycine, aspartate plus asparagine and glutamate plus glutamine with propionate (Fig. 9); alanine with glyoxylate (Fig. 8); alanine and aspartate plus asparagine with α-ketoglutarate (Fig. 9); and glutamate plus glutamine with malate (Fig. 9).

4.3.1 Results Summary - Series I

The total of reported amino acids released by rumen papillae into the incubation medium in the presence of added organic acids was between 20 and 37 μmoles per gram dry papillae per incubation period. When the

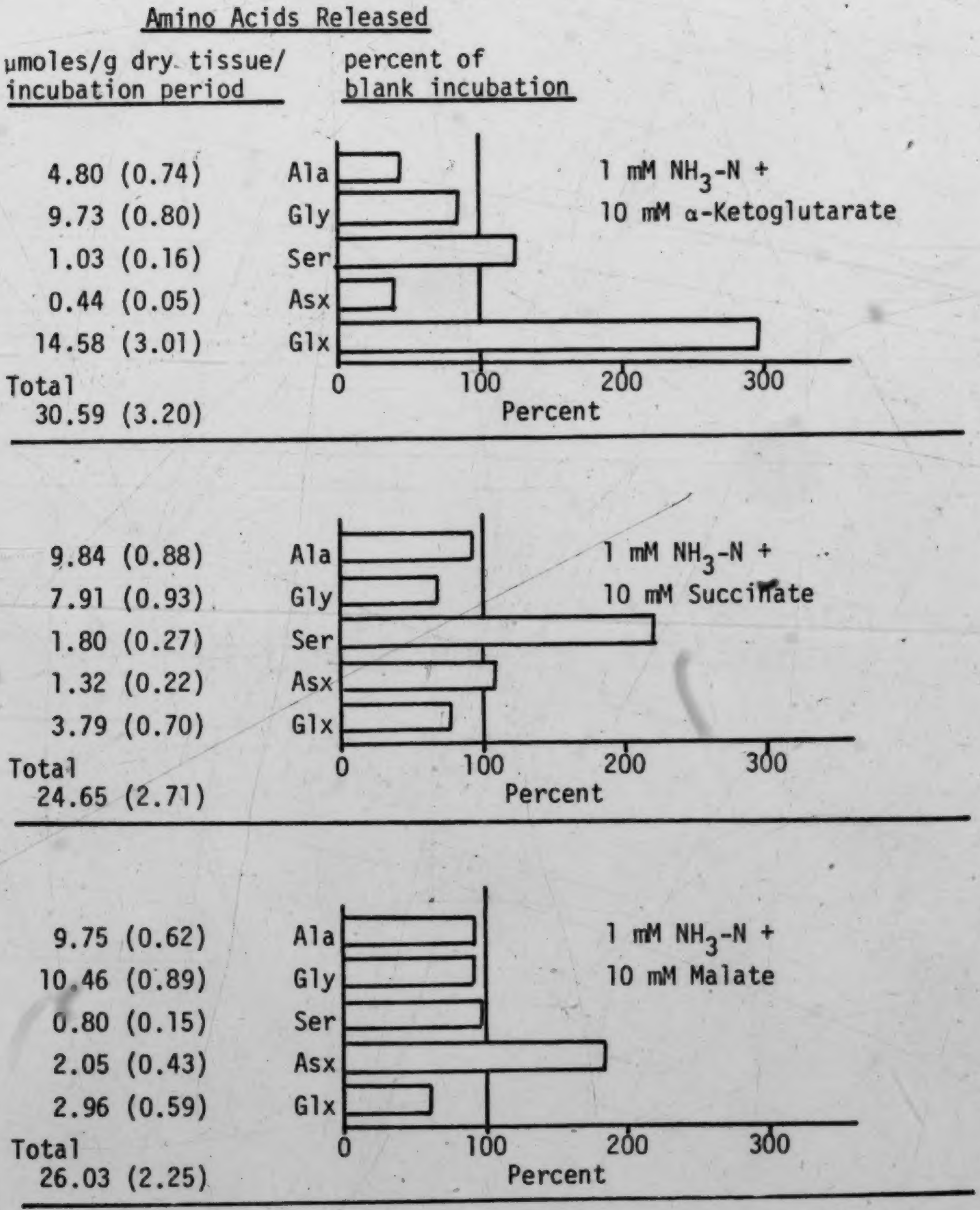


Fig.9. Series I. Amino acid release by rumen papillae incubated with ammonia nitrogen plus α-ketoglutarate, succinate or malate. (Blank incubation Fig.7; mean followed by standard error in parentheses)

addition of an organic acid increased the release of one amino acid relative to the blank, some or all of the other amino acids were released to a lesser extent than in the blank incubation. Glyoxylate was the only organic acid to increase the release of both glycine and serine when compared to the blank incubation.

Alanine and glycine each represented approximately 40% of the reported amino acids present in the blank treatment upon completion of incubation (Table 10). Alanine was increased by the presence of pyruvate or propionate (Fig. 8); glycine was increased by the presence of glyoxylate (Fig. 8). Of the amino acids released by rumen papillae, serine and aspartate plus asparagine were not quantitatively major products. Serine release was increased by the presence of pyruvate, propionate, glyoxylate and succinate (Figs. 8 and 9). Excluding the incubation with α-ketoglutarate, glutamate plus glutamine release by rumen papillae was always less than for the blank or the NH_3-N + 0 incubation (Figs. 7, 8 and 9).

4.4 Series II. Amino Donors

The intent of the treatments of Series II was to assess the effectiveness of alanine as an amino donor for the formation of glycine from glyoxylate and to assess glutamate or glutamine as amino donors with glyoxylate and pyruvate as carbon substrates for the synthesis of glycine and alanine respectively.

4.4.1 Alanine as an Amino Donor With Glyoxylate

Incubating 1 mM alanine with 10 mM glyoxylate (Fig. 10) increased the release of glycine 13.9% over an incubation of 1 mM glutamate plus 10 mM glyoxylate (Fig. 11). Glycine was 333% of the blank incubation, while

Amino Acids Released

Blank Incubation (0 + 0)

	µmoles/g dry tissue/ incubation period
Ala	5.41 (1.92)
Gly	9.10 (1.40)
Ser	0.69 (0.11)
Asx	1.01 (0.12)
Glx	3.07 (0.45)
Total	19.28 (3.78)

1 mM NH_3-N + 0

	µmoles/g dry tissue/ incubation period
Ala	5.26 (1.78)
Gly	9.83 (0.73)
Ser	0.68 (0.06)
Asx	1.02 (0.06)
Glx	3.04 (0.30)
Total	19.83 (2.76)

1 mM Alanine + 10 mM Glyoxylate

	µmoles/g dry tissue/ incubation period
Ala	49.97 (3.37)
Gly	30.27 (3.89)
Ser	2.64 (0.21)
Asx	1.29 (0.19)
Glx	3.72 (0.28)

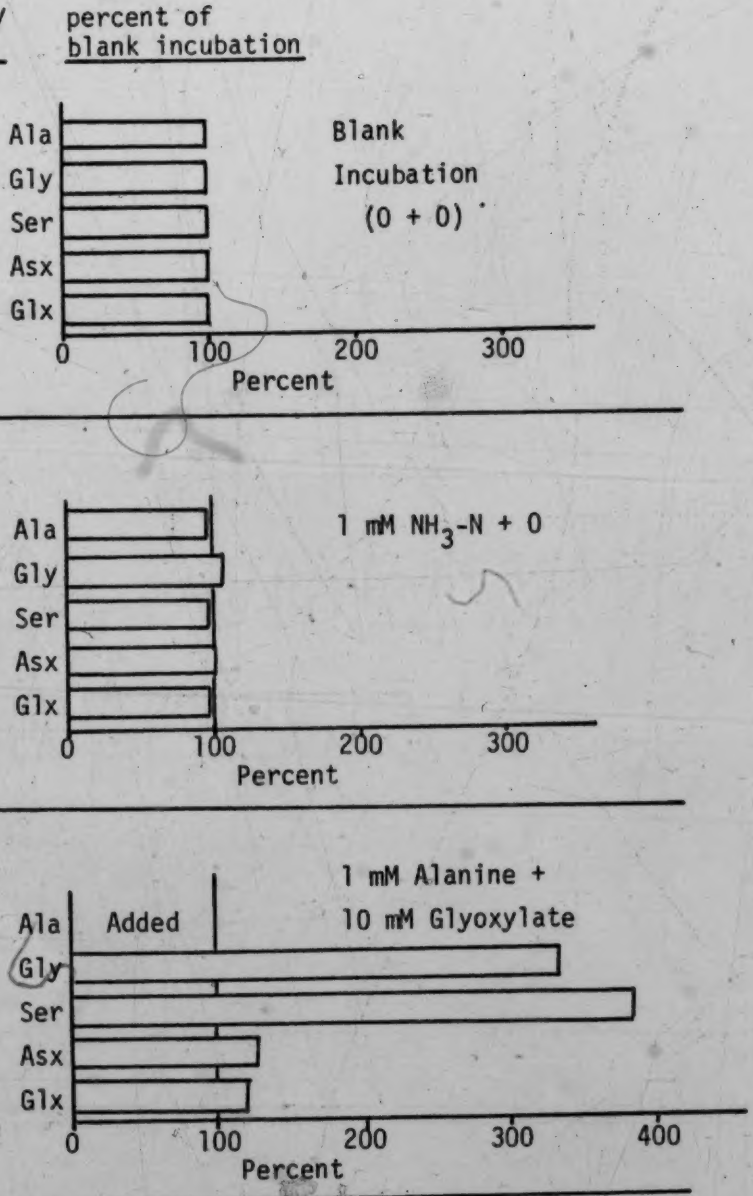


Fig.10. Series II. Amino acid release by rumen papillae incubated with alanine as the nitrogen source plus glyoxylate as the carbon source. (Mean followed by standard error in parentheses)

serine was 383% of the blank with alanine as a nitrogen source and glyoxylate as a carbon source (Fig. 10).

4.4.2 Glutamate and Glutamine as Amino Donors

A series of four incubations were conducted in which α-ketoglutarate plus NH_3-N, glutamate, glutamate plus NH_3-N or glutamine were included with organic acids. An incubation with α-ketoglutarate plus NH_3-N was anticipated to yield glutamate; with glutamate, a direct utilization of glutamate as an amino donor was assessed; with glutamate plus NH_3-N, glutamine was expected to be synthesized yielding a combination of glutamate and glutamine; with glutamine in the incubation medium, the potential of glutamine as an amino donor was assessed. The effectiveness of an amino acid as an amino donor was measured by the quantity of glycine released when glyoxylate was included in the incubation medium, or alternately, by the quantity of alanine released when pyruvate or propionate was in the incubation medium.

In Series II incubations, whenever 1 mM glutamate was added to incubation media, the total glutamate plus glutamine present at the end of the incubation period accounted for approximately 55% of the glutamate added per incubation (Table 11). At the end of the incubation period, the total glutamate plus glutamine present in treatments with added 1 mM glutamine, with 10 mM glyoxylate, pyruvate or propionate as carbon sources for the synthesis of amino acids, accounted for no more than 3% of the glutamine added per incubation (Table 11).

Table 11. Series II. The recovery of glutamate or glutamine added to incubation media as total glutamate plus glutamine present after 3 hours of incubation.

Carbon Source[1]	Nitrogen Source[2]		
	Glutamate	Glutamate + NH_3-N	Glutamine
Glyoxylate	2.58 (0.11)[3]	2.34 (0.09)	0.19 (0.01)
Pyruvate	1.94 (0.21)	1.99 (0.05)	0.08 (0.01)
Propionate	2.27 (0.19)	------[4]	0.10 (0.02)
Average	2.26 (0.19)	2.17 (0.09)	0.12 (0.02)
Number of Observations	9	6	9
Recovered[5] Glu + Gln as a % of added Glu or Gln	56.5	54.3	3.0

1. The concentration of carbon source was 10 mM.
2. The concentration of glutamate, glutamine and NH_3-N was 1 mM. This corresponded to 4 µmoles of glutamate or glutamine per incubation flask before incubation.
3. µmoles of glutamate plus glutamine present in incubation media after 3 hours of incubation. Mean followed by standard error in parentheses.
4. A treatment of 1 mM glutamate plus 1 mM NH_3-N plus 10 mM propionate was not prepared in the treatments of Series II.
5. Recovered total glutamate plus glutamine (Glu + Gln) calculated as a percent of initial glutamate (Glu) or glutamine (Gln) added to each incubation flask.

4.4.2.1 Amino Donors With Glyoxylate

The pattern of amino acid release by rumen papillae with glyoxylate as the carbon substrate were similar for α-ketoglutarate plus NH_3-N and for glutamine and if aspartate plus asparagine were excluded, for glutamate and glutamate plus NH_3-N (Fig. 11). Serine was increased in all four incubations to 375 to 400% of the blank. Glycine was increased to 200% of the blank with α-ketoglutarate plus NH_3-N or glutamine and to 290% of the blank with glutamate or glutamate plus NH_3-N. This was in agreement with a threefold increase for serine and a twofold increase for glycine in the NH_3-N plus glyoxylate treatment of Series I (Fig. 8). The quantities of aspartate plus asparagine released during the incubation period (Fig. 11) were 192% and 142% of the blank with added glutamate and glutamate plus NH_3-N respectively.

4.4.2.2 Amino Donors With Pyruvate

The patterns of release for all amino acids were similar with either α-ketoglutarate plus NH_3-N or glutamine in the incubation medium; with either glutamate or glutamate plus NH_3-N, the patterns of release of all amino acids except alanine were similar (Fig. 12). Alanine release was increased to approximately 170% of the blank upon the inclusion of α-ketoglutarate plus NH_3-N, 190% of the blank with glutamine, 560% of the blank with glutamate and 720% of the blank with glutamate plus NH_3-N. The difference for alanine between glutamate and glutamate plus NH_3-N was within the standard error of the observations. The concentrations of glycine (75 to 90% of the blank) and serine (approximately 200% of the blank) did not differ among substrate treatments. For aspartate plus asparagine,

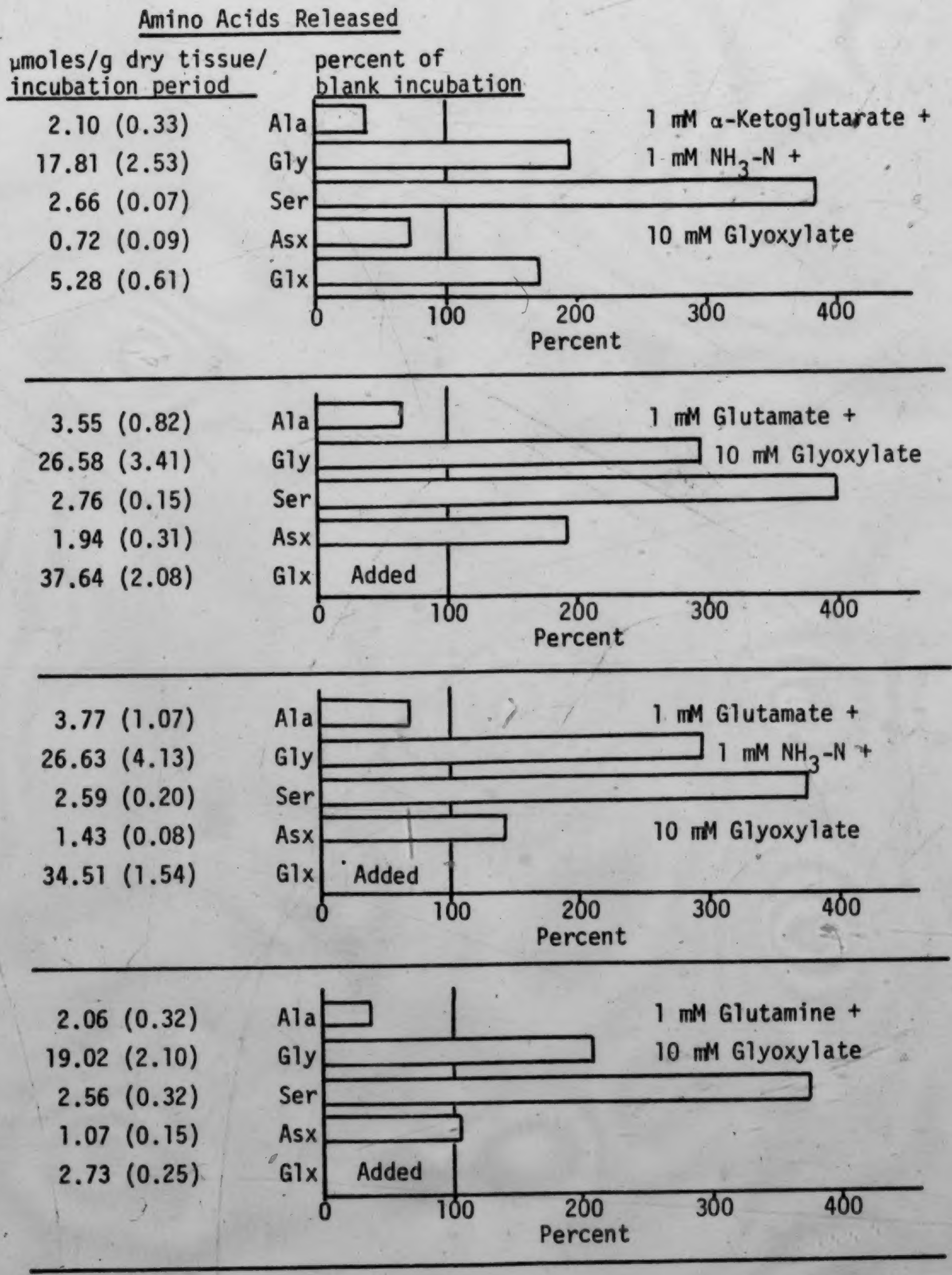


Fig.11. Series II. Amino acid release by rumen papillae incubated with nitrogen sources plus glyoxylate as the major carbon source. (Blank incubation Fig.10; mean followed by standard error in parentheses)

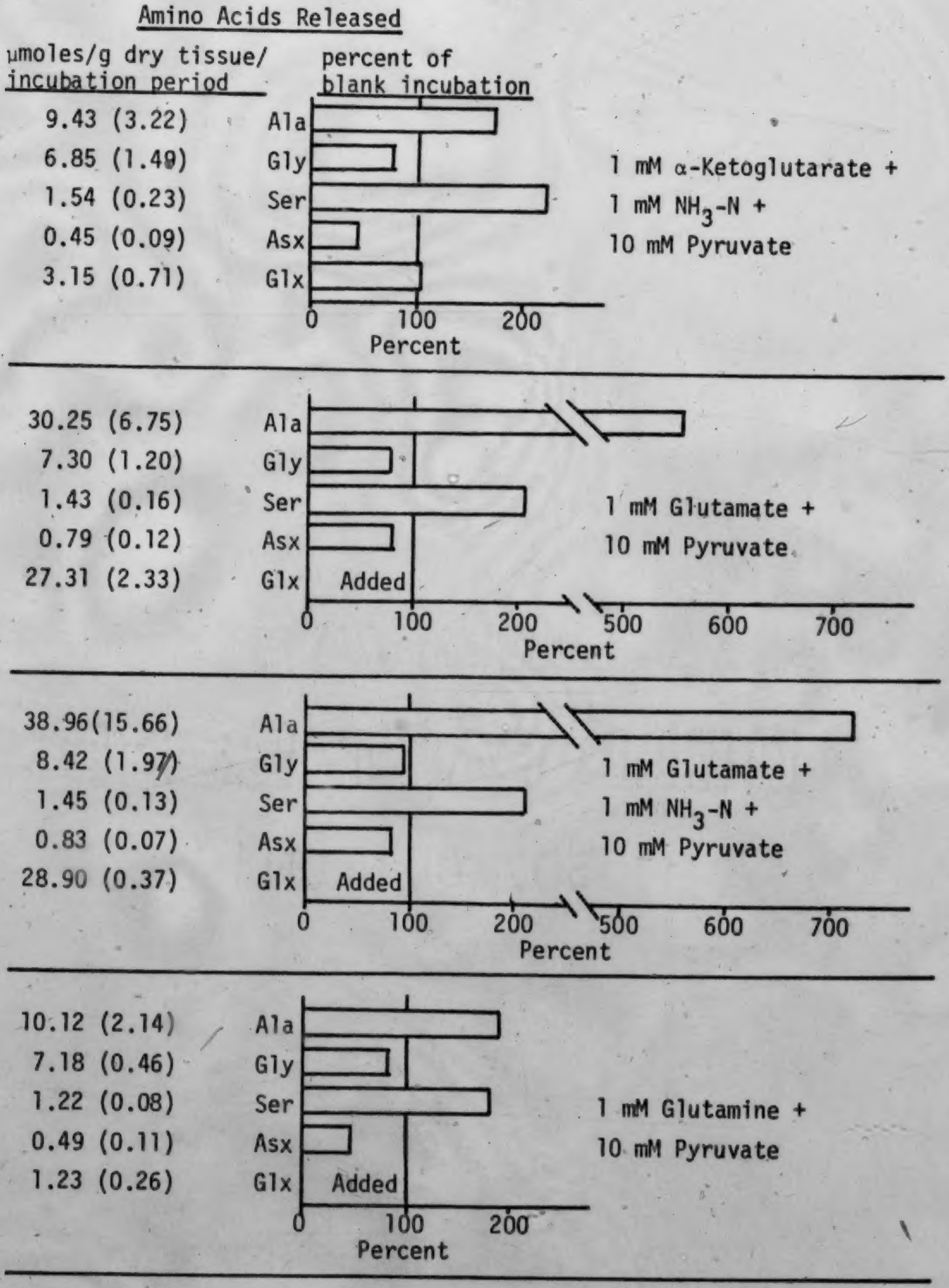


Fig.12. Series II. Amino acid release by rumen papillae incubated with nitrogen sources plus pyruvate as the major carbon source. (Blank incubation Fig.10; mean followed by standard error in parentheses)

with α-ketoglutarate plus NH_3-N or glutamine, release was approximately 45 to 50% of the blank, and with glutamate or glutamate plus NH_3-N, release was approximately 80% of the blank.

4.4.2.3 Amino Donors With Propionate

The quantities of alanine and glycine present at the completion of incubation were less with propionate (Fig. 13) as the added carbon substrate than with pyruvate (Fig. 12). Final serine levels were similar with propionate (Fig. 13) and with pyruvate (Fig. 12); final aspartate plus asparagine levels were higher with propionate (Fig. 13) than with pyruvate (Fig. 12) as the carbon source. The quantity of glutamate plus glutamine in the presence of α-ketoglutarate plus NH_3-N was 146% of the blank with propionate (Fig. 13) as compared to 103% of the blank with pyruvate (Fig. 12).

Alanine release into the incubation medium by rumen papillae with propionate present (Fig. 13) was 73%, 275% and 111% of the blank incubation with α-ketoglutarate plus NH_3-N, glutamate, and glutamine respectively; the release of glycine (50% of the blank) and serine (210 to 250% of the blank) did not differ among substrate treatments (Fig. 13). The release of aspartate plus asparagine was greater with glutamate than with α-ketoglutarate plus NH_3-N or glutamine as nitrogen sources in the presence of propionate (Fig. 13).

4.5 Series III Incubations

4.5.1 Glycine from Two-Carbon Compounds

Of the two-carbon compounds studied, including glycolaldehyde, glyoxal, glycollate, glyoxylate and oxalate at 10 mM (Figs. 14 and 15),

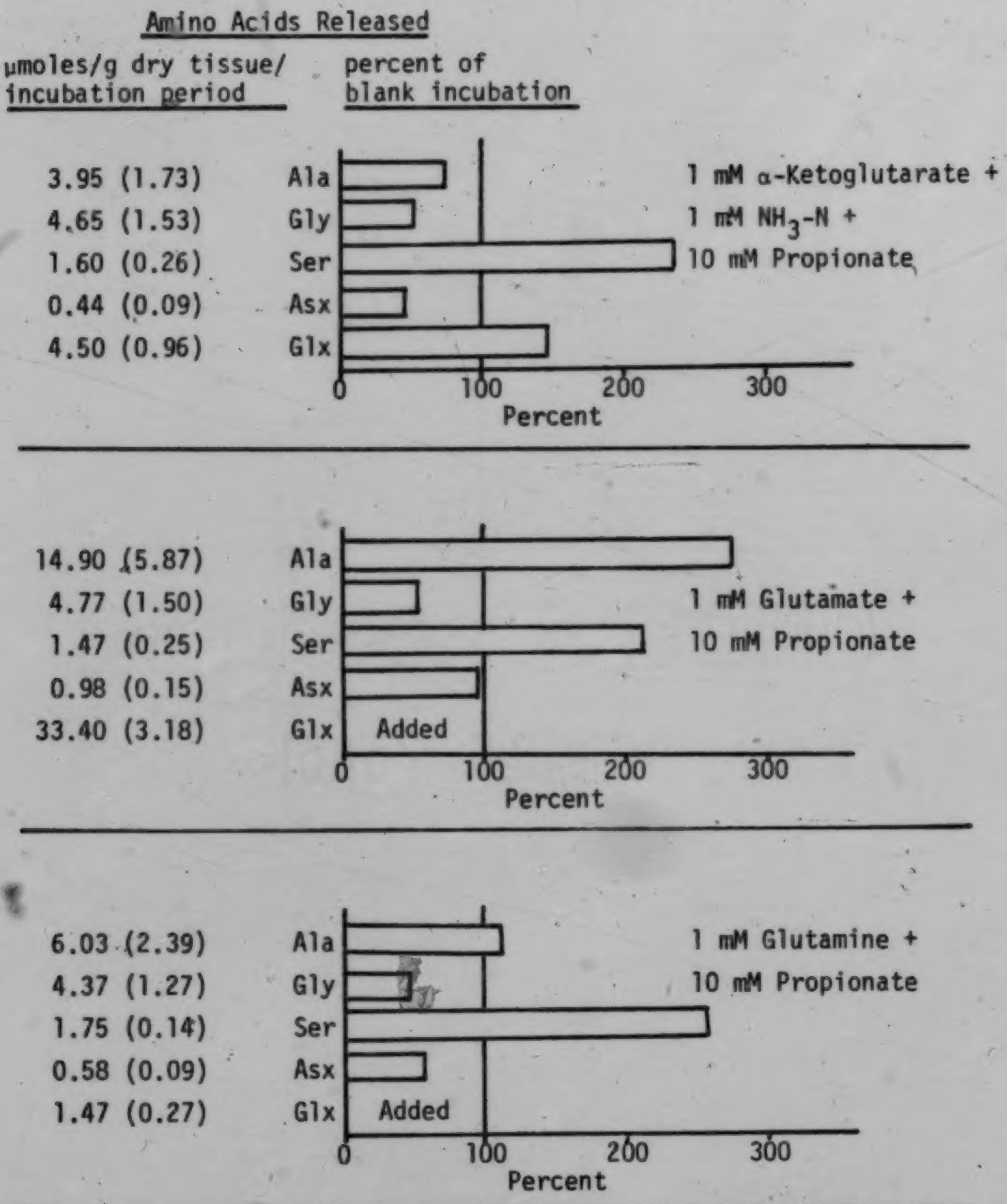


Fig.13. Series II. Amino acid release by rumen papillae incubated with nitrogen sources plus propionate as the major carbon source. (Blank incubation Fig.10; mean followed by standard error in parentheses)

Amino Acids Released

	µmoles/g dry tissue/ incubation period
Blank Incubation (0 + 0)	
Ala	7.95 (1.07)
Gly	8.70 (0.53)
Ser	0.70 (0.11)
Asx	0.90 (0.21)
Glx	3.74 (0.69)
Total	21.99 (3.42)
1 mM NH_3-N + 0	
Ala	8.71 (1.36)
Gly	9.25 (1.48)
Ser	0.70 (0.14)
Asx	0.77 (0.05)
Glx	3.62 (0.58)
Total	23.04 (3.14)
1 mM NH_3-N + 10 mM Glycolaldehyde	
Ala	9.07 (0.98)
Gly	6.97 (0.94)
Ser	0.58 (0.05)
Asx	0.71 (0.10)
Glx	4.81 (0.53)
Total	22.54 (2.43)
1 mM NH_3-N + 10 mM Glyoxal	
Ala	7.64 (1.56)
Gly	8.00 (1.49)
Ser	0.70 (0.11)
Asx	0.70 (0.11)
Glx	3.60 (0.86)
Total	20.65 (4.10)

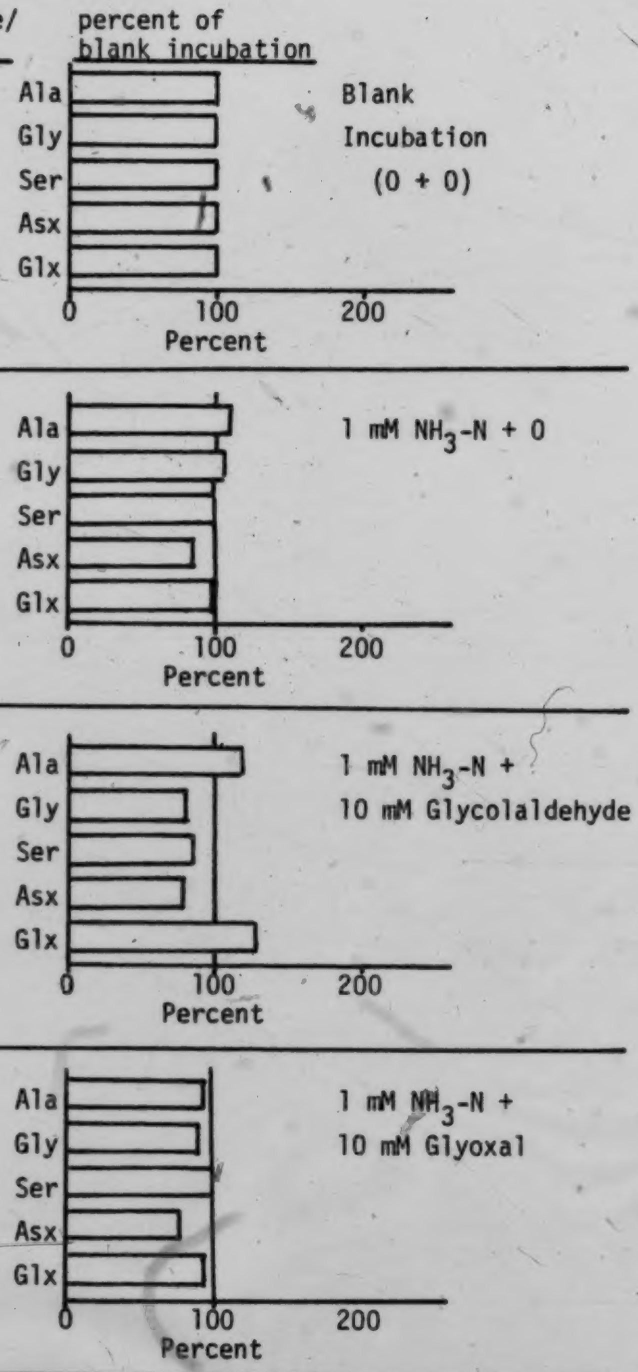


Fig.14. Series III. Amino acid release by rumen papillae incubated with ammonia nitrogen plus the two-carbon compounds glycolaldehyde or glyoxal. (Mean followed by standard error in parentheses)

Amino Acids Released

	µmoles/g dry tissue/ incubation period
Ala	8.88 (1.75)
Gly	9.18 (2.30)
Ser	0.91 (0.13)
Asx	0.91 (0.23)
Glx	3.98 (0.74)
Total	23.87 (5.07)
Ala	3.53 (1.00)
Gly	18.10 (3.42)
Ser	2.20 (0.25)
Asx	0.99 (0.15)
Glx	3.88 (0.57)
Total	28.71 (5.19)
Ala	8.25 (0.91)
Gly	12.98 (1.62)
Ser	1.24 (0.12)
Asx	2.21 (0.53)
Glx	5.23 (0.66)
Total	29.91 (3.12)

percent of blank incubation

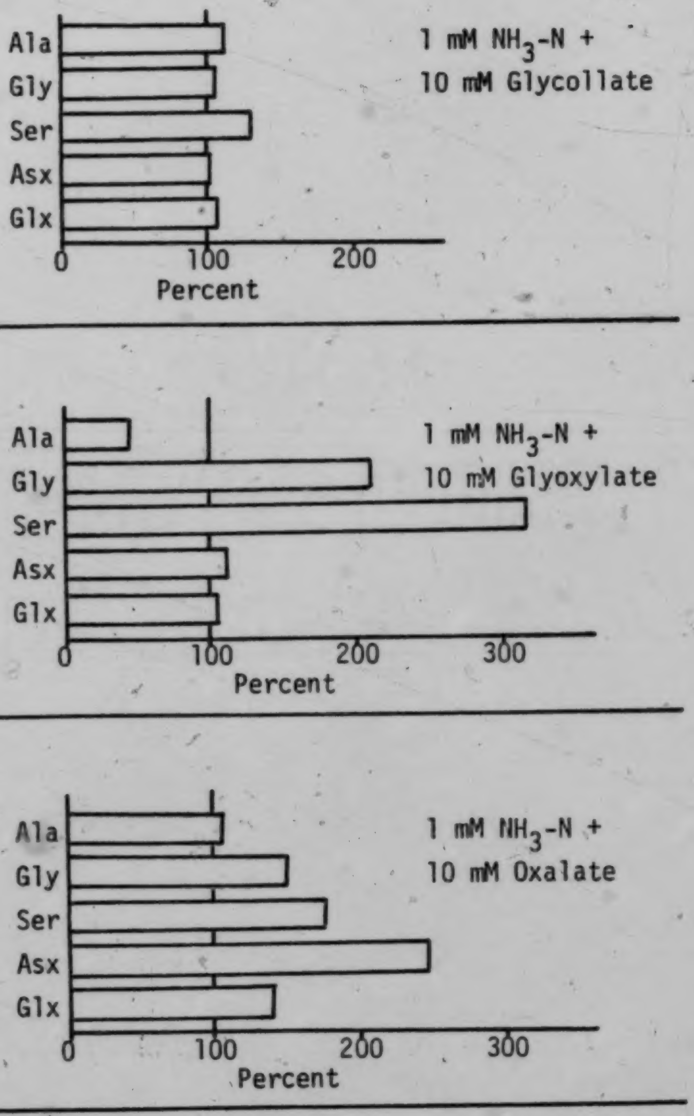


Fig.15. Series III. Amino acid release by rumen papillae incubated with ammonia nitrogen plus the two-carbon compounds glycollate, glyoxylate or oxalate. (Blank incubation Fig.14; mean followed by standard error in parentheses)

glyoxylate increased the release of glycine to 208% of the blank incubation, while oxalate increased glycine to 149% of the blank incubation (Fig. 15). The other two-carbon compounds did not increase release of glycine when compared to the blank incubation (Figs. 14 and 15).

Glycolaldehyde increased the quantity of alanine to 119% of the blank incubation and glutamate plus glutamine to 129% of the blank (Fig. 14). This glutamate plus glutamine release represented 21.4% of total reported amino acids as compared to 16.9% for the blank incubation (Table 12).

Athough the total amino acids in the incubation media were higher in Series I than Series III(Compare the blank incubations, Figs. 7 and 14 respectively), data for glyoxylate incubations calculated as a percent of the blank incubation (Tables 10 and 12 for Series I and III respectively), were quite similar. In the presence of added glyoxylate in Series III (Fig. 15), glycine and serine release by rumen papillae were 208% and 314% of the blank incubation respectively.

Oxalate (10 mM) increased the release of glycine, serine, aspartate plus asparagine and glutamate plus glutamine (149%, 177%, 246% and 140% of the blank incubation respectively), but did not influence alanine release by rumen papillae (Fig. 15).

4.5.2 Glycine from Glyoxylate

Incubations of 0, 5, 10 and 20 mM glyoxylate with rumen papillae increased the release of glycine and serine over the blank incubation (Table 13; Fig. 16). Alanine release was decreased to approximately 25 to 40% of the blank with glyoxylate present, while aspartate plus asparagine and glutamate plus glutamine release were not changed markedly (Table 13).

Table 12. Series III. Amino acids calculated as a percent of the total reported amino acids released by rumen papillae incubated with ammonia nitrogen plus: A. two-carbon compounds; or B. citrate, α-ketoglutarate or glucose.

Treatment	Amino Acid				
	Ala	Gly	Ser	Asx	Glx
Blank Incubation	36.4 (2.4)[1]	39.4 (2.4)	3.2 (0.1)	4.0 (0.6)	16.9 (0.8)
NH_3-N + 0 [2]	37.8 (3.2)	39.9 (1.4)	3.0 (0.5)	3.4 (0.4)	15.8 (1.4)
A. NH_3-N + Glycolaldehyde[3]	42.1 (1.6)	30.7 (0.8)	2.6 (0.1)	3.2 (0.6)	21.4 (0.8)
NH_3-N + Glyoxal	36.9 (1.3)	39.0 (1.0)	3.5 (0.3)	3.5 (0.3)	17.2 (0.8)
NH_3-N + Glycollate	37.4 (1.6)	37.9 (1.4)	3.9 (0.3)	3.9 (0.8)	16.9 (0.5)
NH_3-N + Glyoxylate	11.8 (1.6)	62.8 (1.9)	8.0 (1.1)	3.6 (0.6)	13.9 (1.6)
NH_3-N + Oxalate	27.8 (3.0)	43.3 (1.8)	4.2 (0.4)	7.3 (1.4)	17.4 (0.6)
B. NH_3-N + Citrate	28.5 (2.0)	38.6 (1.6)	3.4 (0.3)	3.3 (0.5)	26.3 (2.6)
NH_3-N + α-Ketoglutarate	13.4 (3.7)	30.5 (4.0)	4.2 (0.1)	1.7 (0.1)	50.2 (7.6)
NH_3-N + Glucose	46.0 (1.8)	25.0 (1.8)	6.6 (0.9)	2.6 (0.3)	19.8 (3.5)

1. Mean (n=3) followed by standard error in parentheses.
2. The concentration of NH_3-N for all treatments was 1 mM.
3. The concentration of carbon source in all treatments was 10 mM.

Table 13. Series III. Amino acid release by rumen papillae incubated with: A. ammonia nitrogen plus 0, 5, 10 and 20 mM glyoxylate; and B. 0, 1, 3, 5 and 10 mM ammonia nitrogen.

Treatment	Amino Acid[1]					Total
	Ala	Gly	Ser	Asx	Glx	
A. 1 mM Ammonia Nitrogen plus						
No Glyoxylate [2]	8.71 (1.36)[3]	9.25 (1.48)	0.70 (0.14)	0.77 (0.05)	3.62 (0.58)	23.04 (3.14)
5 mM Glyoxylate	3.42 (0.76)	14.74 (2.33)	1.61 (0.33)	0.65 (0.15)	2.96 (0.42)	23.38 (3.98)
10 mM Glyoxylate	3.53 (1.00)	18.10 (3.42)	2.20 (0.25)	0.90 (0.15)	3.88 (0.57)	28.71 (5.19)
20 mM Glyoxylate	2.33 (0.48)	21.52 (2.82)	2.04 (0.33)	0.80 (0.14)	3.82 (0.77)	30.56 (4.35)
B. Ammonia Nitrogen (mM)						
0 [4]	7.95 (1.07)	8.70 (0.53)	0.70 (0.11)	0.90 (0.21)	3.74 (0.69)	21.99 (3.42)
1	8.71 (1.36)	9.25 (1.48)	0.70 (0.14)	0.77 (0.05)	3.62 (0.58)	23.04 (3.14)
3	8.26 (1.40)	9.39 (1.80)	0.69 (0.07)	0.74 (0.11)	3.78 (0.50)	22.87 (3.60)
5	7.33 (1.30)	8.51 (1.63)	0.64 (0.08)	0.72 (0.06)	3.33 (0.35)	20.52 (3.06)
10	7.11 (1.23)	9.19 (1.27)	0.69 (0.07)	0.70 (0.07)	3.51 (0.27)	21.20 (2.73)

1. µmoles of amino acid per gram dry rumen papillae per incubation period.
2. The 1 mM ammonia nitrogen plus no glyoxylate treatment was the 1 mM NH_3-N + 0 incubation of Series III in Fig.14.
3. Mean (n=3) followed by standard error in parentheses.
4. This incubation was the blank incubation of Series III in Fig.14.

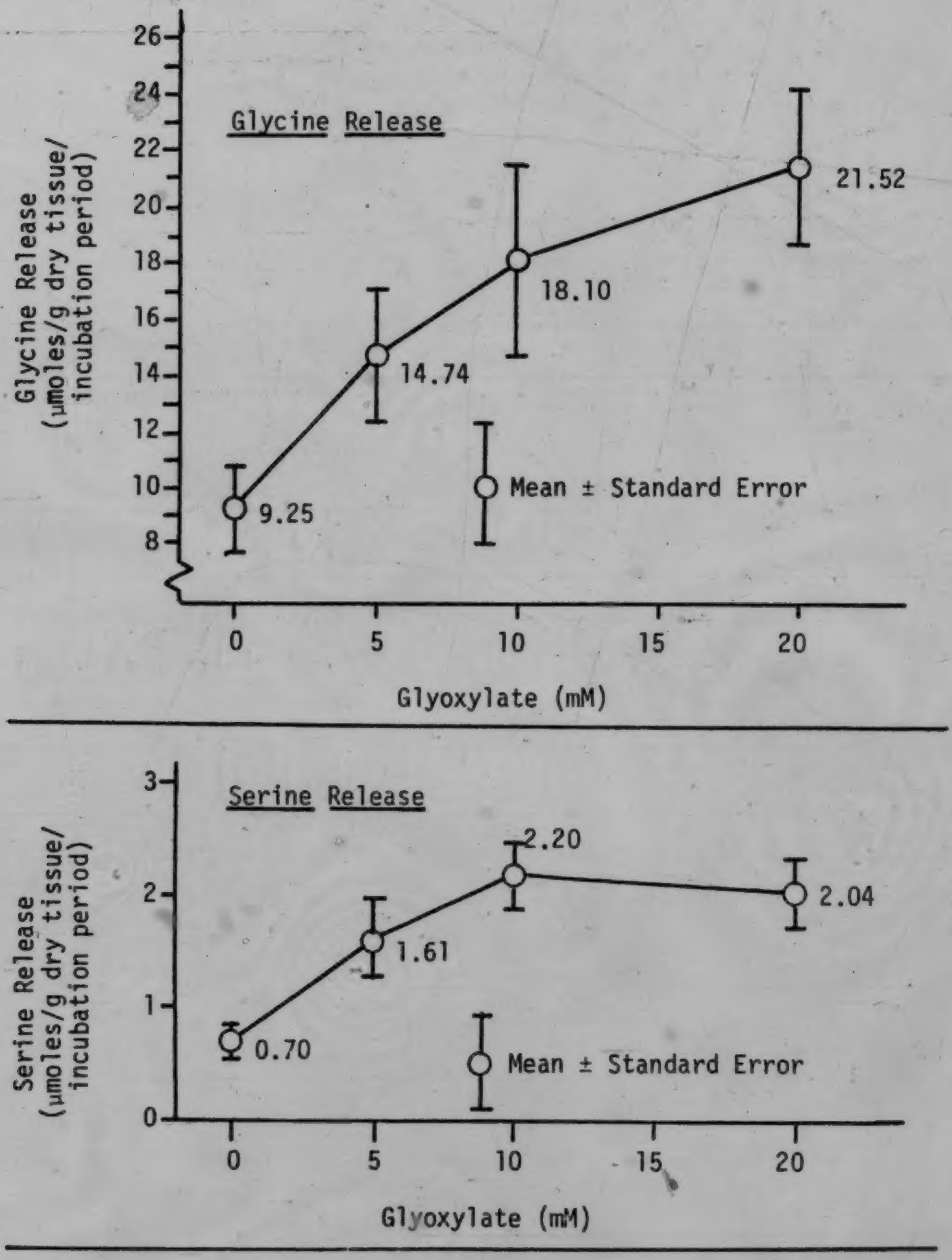


Fig.16. Series III. Glycine and serine release by rumen papillae incubated with ammonia nitrogen plus 0, 5, 10 and 20 mM glyoxylate.

There were curvilinear relationships of glycine release and of serine release with initial glyoxylate concentrations (Fig. 16).

4.5.3 Incubations With Ammonia Nitrogen

Increasing the concentration of NH_3-N up to 10 mM (0, 1, 3, 5 and 10 mM) did not change the total quantity or spectrum of amino acids in the incubation medium (Table 13).

4.5.4 Ammonia Nitrogen Plus Citrate, α-Ketoglutarate or Glucose

The release of glutamate plus glutamine in the presence of NH_3-N was increased by the addition of citrate or α-ketoglutarate to the incubation medium (Fig. 17). Glutamate plus glutamine in the presence of 10 mM α-ketoglutarate was 292% of the blank and in the presence of citrate was 164% of the blank. Citrate also had less influence upon the release of other amino acids, particularly alanine and aspartate plus asparagine, when compared to the blank, than did α-ketoglutarate. The release of serine by rumen papillae was 117% and 134% of the blank with citrate and α-ketoglutarate respectively.

The inclusion of 10 mM glucose as a substrate resulted in an accumulation of less total amino acids in the incubation medium (16.10 μmoles per gram dry papillae per incubation period; Fig. 17), than occurred in the blank incubation (21.99 μmoles per gram dry papillae per incubation period; Fig. 14). Alanine represented 46.0% of total reported amino acids with glucose as contrasted to 36.4% in the blank (Table 12). Serine was 155% of the blank incubation in the presence of added glucose (Fig. 17). Incubation of 10 mM glucose with rumen papillae resulted in the release of

Amino Acids Released

	µmoles/g dry tissue/ incubation period
Ala	6.95 (1.42)
Gly	9.41 (2.00)
Ser	0.82 (0.19)
Asx	0.77 (0.03)
Glx	6.15 (0.09)
Total	24.10 (4.18)

Ala	3.07 (1.18)
Gly	6.92 (1.71)
Ser	0.94 (0.15)
Asx	0.38 (0.07)
Glx	10.93 (1.65)
Total	22.25 (3.12)

Ala	7.47 (1.56)
Gly	4.10 (0.96)
Ser	1.09 (0.34)
Asx	0.44 (0.13)
Glx	3.00 (0.33)
Total	16.10 (3.14)

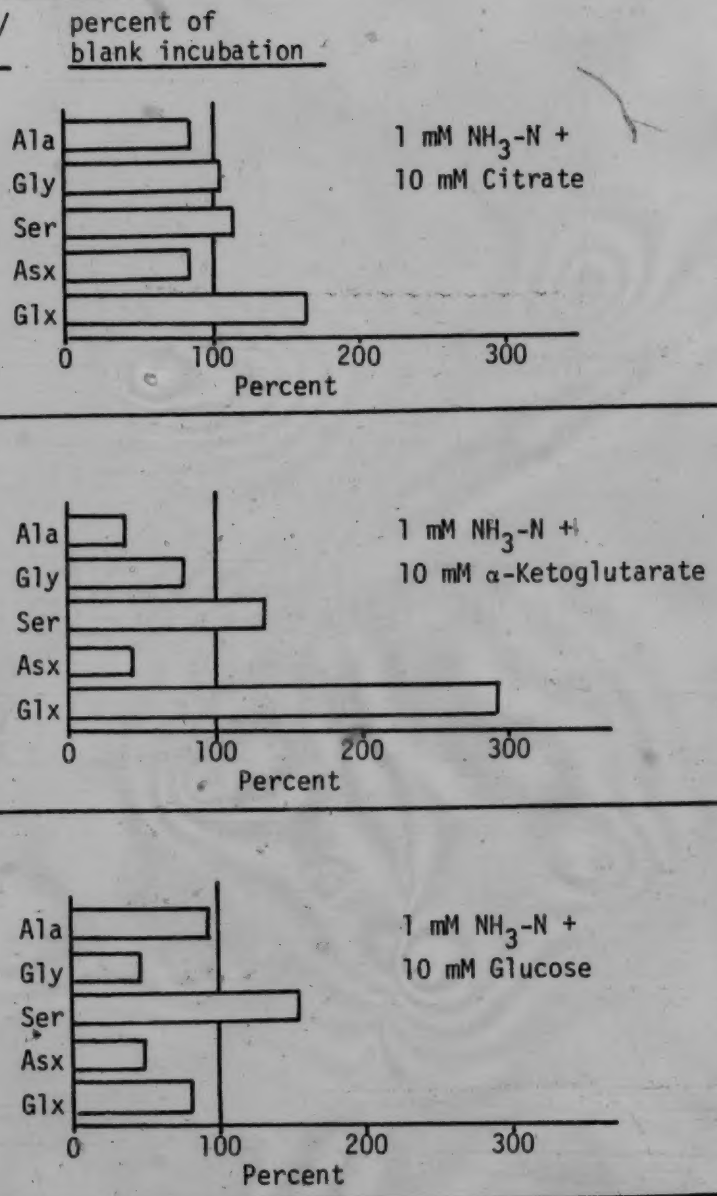


Fig.17. Series III. Amino acid release by rumen papillae incubated with ammonia nitrogen plus citrate, α-ketoglutarate or glucose. (Blank incubation Fig.14; mean followed by standard error in parentheses)

glycine and aspartate plus asparagine to approximately 50% of the blank incubation (Fig. 17).

4.6 Series IV. Glycine-Serine Interrelationships

4.6.1 Serine Incubations

Serine was incubated with rumen papillae at initial concentrations of 0, 0.1, 0.5, 1.0 and 2.0 mM (Table 14). The change of glycine recovery in incubation media, in response to added serine was the largest between 0.1 and 0.5 mM serine (Fig. 18). Above 0.5 mM serine, the release of glycine by rumen papillae did not change markedly. The presence of serine in the incubation medium did not influence alanine, aspartate plus asparagine or glutamate plus glutamine release by rumen papillae (Table 14).

4.6.2 Glycine Incubations

Glycine was incubated with rumen papillae at initial concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mM (Table 14). Serine release by rumen papillae did not respond to increased concentrations of glycine in the incubation medium (Fig. 19). Alanine, aspartate plus asparagine and glutamate plus glutamine were not influenced by the presence of added glycine (Table 14).

4.7 Series V. Amino Donors

4.7.1 Glutamate Versus Glutamine, Methionine Sulfoximine Preparations

In Series V, as in Series II, the relative importance of glutamate or glutamine as an amino donor was investigated. Methionine sulfoximine (MS), a non-competitive inhibitor of glutamine synthetase (Meister 1969),

Table 14. Series IV. Amino acid release by rumen papillae incubated with serine or glycine.

Treatment	Amino Acid[1]				
	Ala	Gly	Ser	Asx	Glx
Blank Incubation	6.73 (1.18)[2]	8.59 (0.94)	0.94 (0.12)	0.83 (0.07)	4.25 (0.09)
Serine (mM)					
0.1	6.39 (0.44)	8.84 (0.79)	4.57 (0.20)	0.77 (0.06)	5.06 (0.41)
0.5	7.46 (0.73)	12.80 (0.37)	21.41 (1.29)	0.87 (0.06)	5.27 (0.36)
1.0	6.08 (1.82)	13.63 (0.36)	47.00 (5.24)	0.90 (0.12)	5.70 (0.86)
2.0	6.48 (1.18)	15.88 (2.07)	86.86 (9.07)	0.85 (0.04)	4.67 (0.50)
Glycine (mM)					
0.25	6.98 (0.95)	22.66 (0.81)	0.97 (0.08)	0.89 (0.10)	4.75 (0.34)
0.5	6.56 (1.39)	35.44 (2.68)	0.76 (0.06)	0.85 (0.06)	4.57 (0.36)
1.0[3]	6.71 (--)	66.89 (--)	0.92 (--)	0.90 (--)	4.29 (--)
2.0	5.95 (1.00)	122.25 (9.34)	0.85 (0.17)	0.81 (0.03)	4.01 (0.17)
4.0	7.03 (0.95)	226.51(12.10)	0.85 (0.20)	0.94 (0.09)	4.30 (0.20)

1. μmoles of amino acid per gram dry rumen papillae per incubation period.
2. Mean (n=3) followed by standard error in parentheses.
3. For incubations with 1 mM glycine n=2.

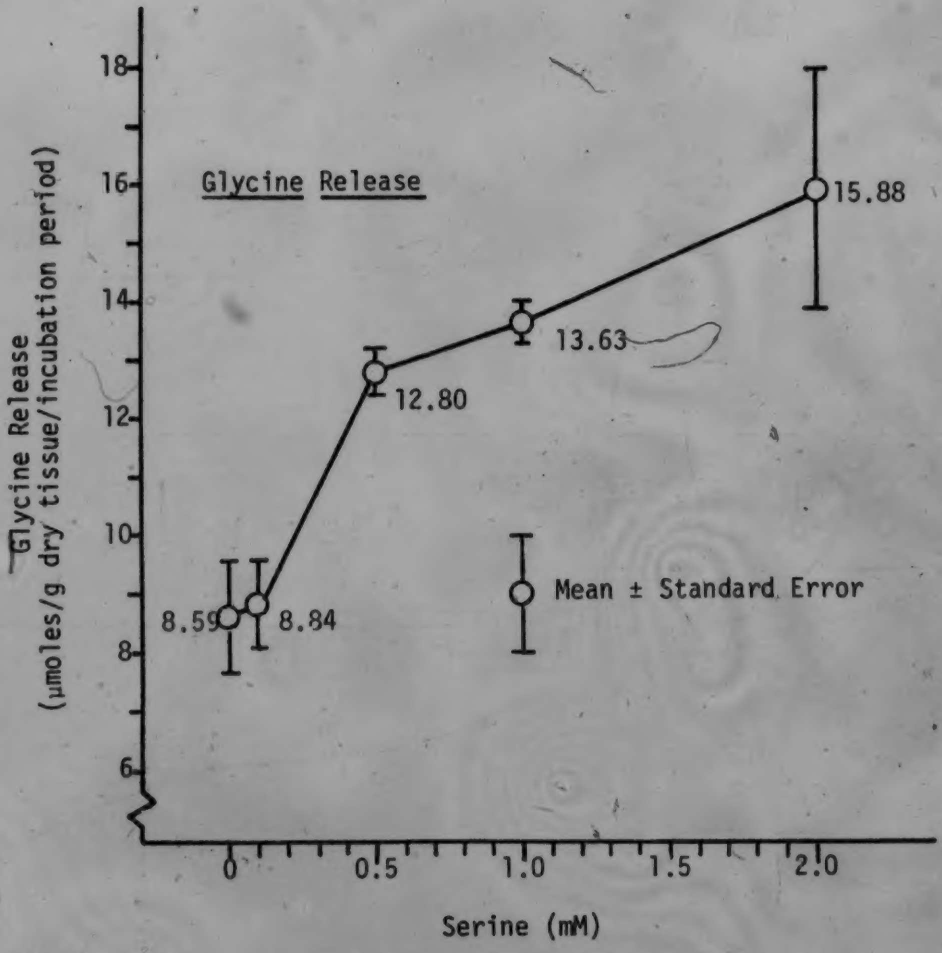


Fig.18. Series IV. Glycine release by rumen papillae incubated with serine.

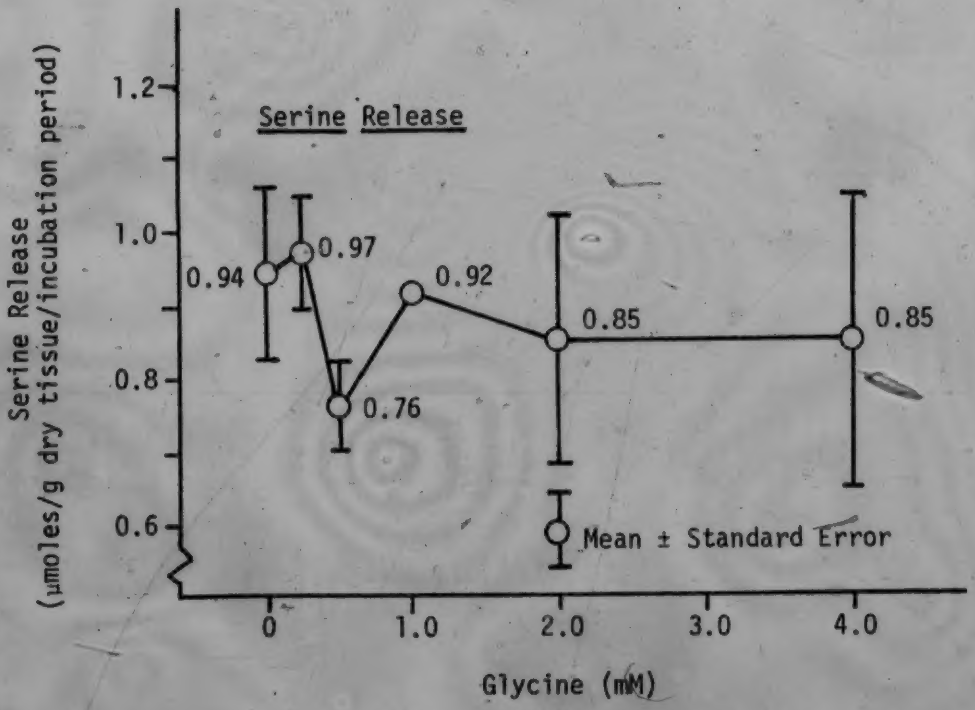


Fig.19. Series IV. Serine release by rumen papillae incubated with glycine. (n=2 at 1 mM glycine)

was added to incubation media to prevent the formation of glutamine.

Only when no added glutamate was present (Fig. 20), was the influence of MS upon glutamate plus glutamine release by rumen papillae indicated. Addition of MS reduced glutamate plus glutamine by 0.33 and 0.78 μmoles per g dry papillae per incubation period for incubations without and with added NH_3-N respectively (Fig. 20); these suggestions of change did not exceed the range of standard errors of the measurements. With no added glutamate or carbon sources, there was no effect of added MS upon glycine, serine or aspartate plus asparagine release by rumen papillae. Alanine was 133% and 128% of the blank for the NH_3-N + 0 and the blank incubations respectively when MS was added to these incubations (Fig. 20).

The amino acids released by rumen papillae incubated with glutamate plus pyruvate (Fig. 21) and with glutamate plus glyoxylate (Fig. 22) were not influenced by the addition of MS to incubation media.

4.7.2 Alanine Plus Glyoxylate With Hydroxylamine

The results of Series II indicated that incubation of alanine with glyoxylate (Fig. 10) increased the release of glycine to a larger extent than did an incubation of glutamate with glyoxylate. The alanine incubations of Series V (Fig. 23) were undertaken to obtain additional information on the Series II observation. Hydroxylamine, an inhibitor of alanine-glyoxylate aminotransferase from human liver (Thompson and Richardson 1967), was used with the intention of preventing the formation of glycine from alanine and glyoxylate.

The level of glycine in the incubation medium was increased to 122% of the blank when 1 mM alanine alone was added to the incubation medium,

Amino Acids Released

μmoles/g dry tissue/ incubation period

	Blank Incubation (0 + 0)	0 + 0 + 1 mM Methionine Sulfoximine	1 mM NH_3-N + 0	1 mM NH_3-N + 0 + 1 mM Methionine Sulfoximine
Ala	6.61 (0.79)	8.82 (0.65)	6.94 (0.27)	8.10 (0.54)
Gly	9.85 (0.97)	10.68 (0.41)	10.19 (0.63)	10.60 (0.21)
Ser	1.07 (0.07)	1.08 (0.10)	1.12 (0.05)	0.92 (0.02)
Asx	0.95 (0.38)	0.94 (0.34)	0.90 (0.33)	1.01 (0.30)
Glx	3.92 (0.53)	3.59 (0.30)	4.02 (0.50)	3.24 (0.30)
Total	22.40 (0.94)	21.53 (1.33)	19.16 (0.71)	20.63 (0.34)

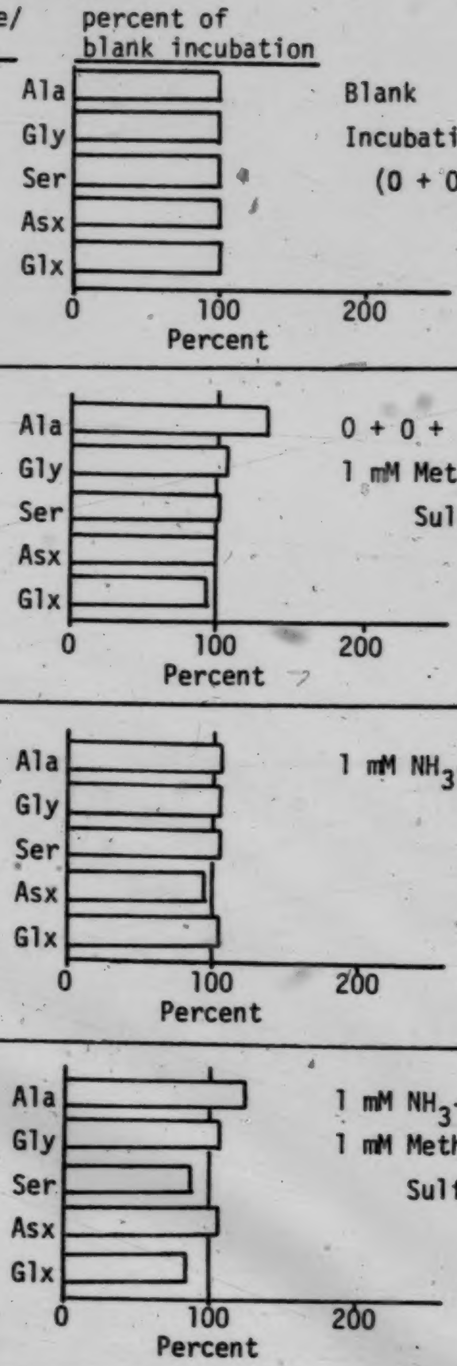


Fig.20. Series V. Amino acid release by rumen papillae incubated in the presence of methionine sulfoximine, with and without ammonia nitrogen. (Mean followed by standard error in parentheses)

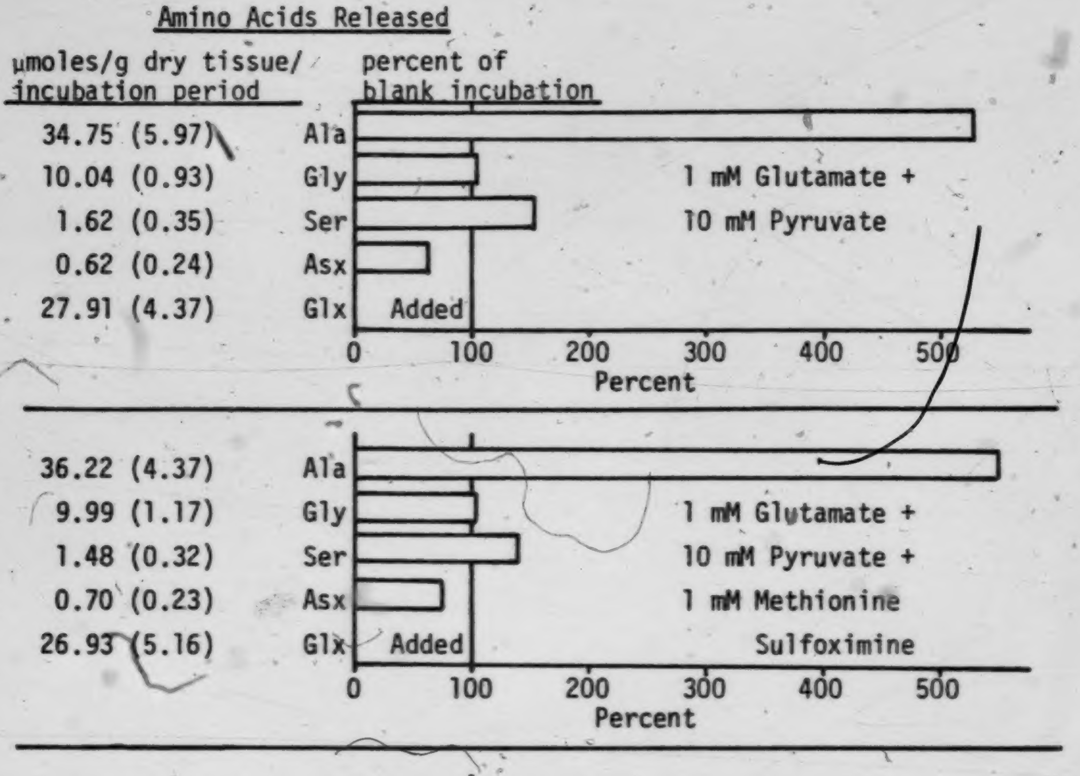


Fig.21. Series V. Amino acid release by rumen papillae incubated with glutamate plus pyruvate in the presence of methionine sulfoximine, with and without ammonia nitrogen. (Blank incubation Fig.20; mean followed by standard error in parentheses)

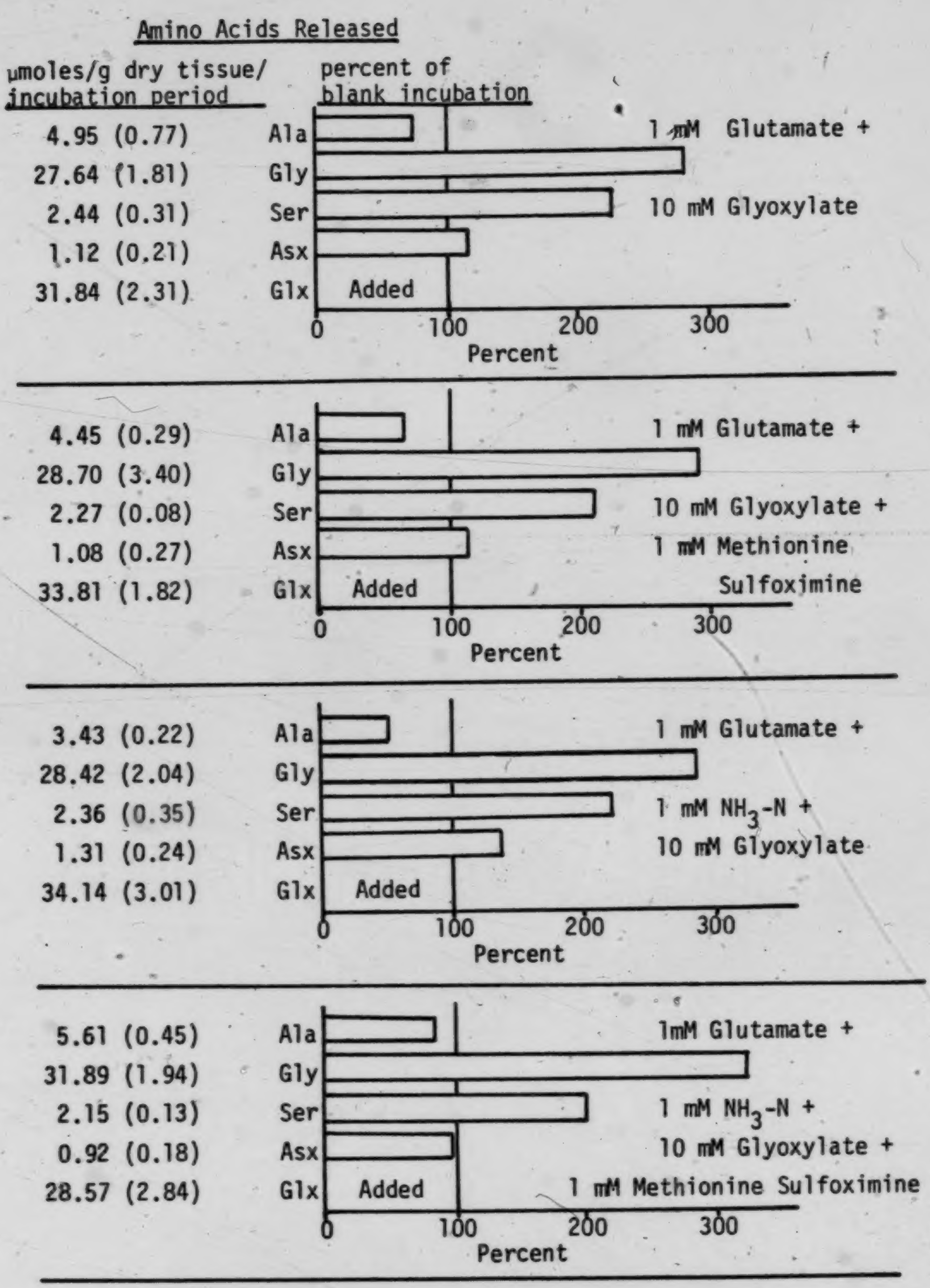


Fig.22. Series V. Amino acid release by rumen papillae incubated with glutamate plus glyoxylate in the presence of methionine sulfoximine, with and without ammonia nitrogen. (Blank incubation Fig.20; mean followed by standard error in parentheses)

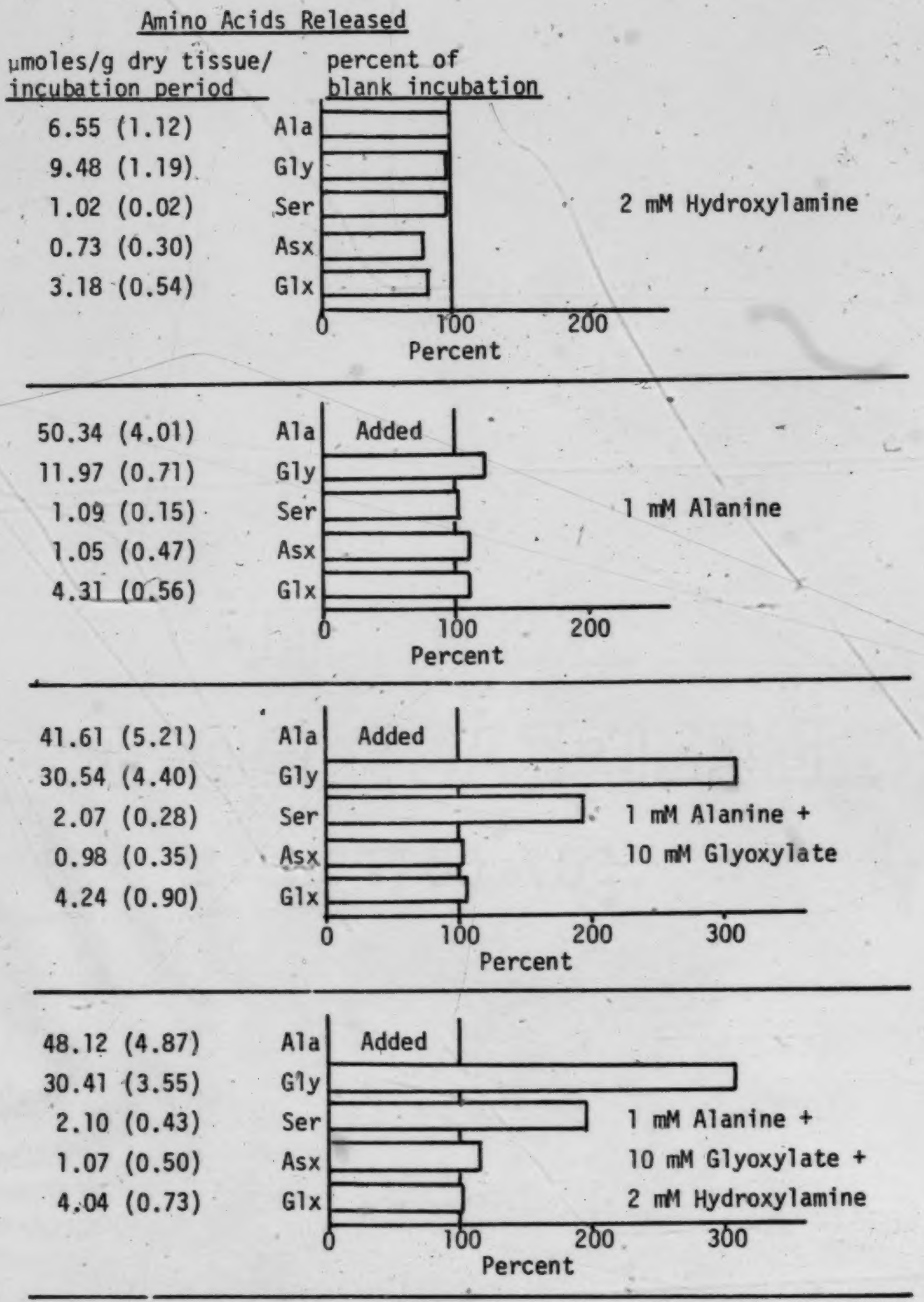


Fig.23. Series V. Amino acid release by rumen papillae incubated with alanine plus glyoxylate, with and without hydroxylamine. (Blank incubation Fig.20; mean followed by standard error in parentheses)

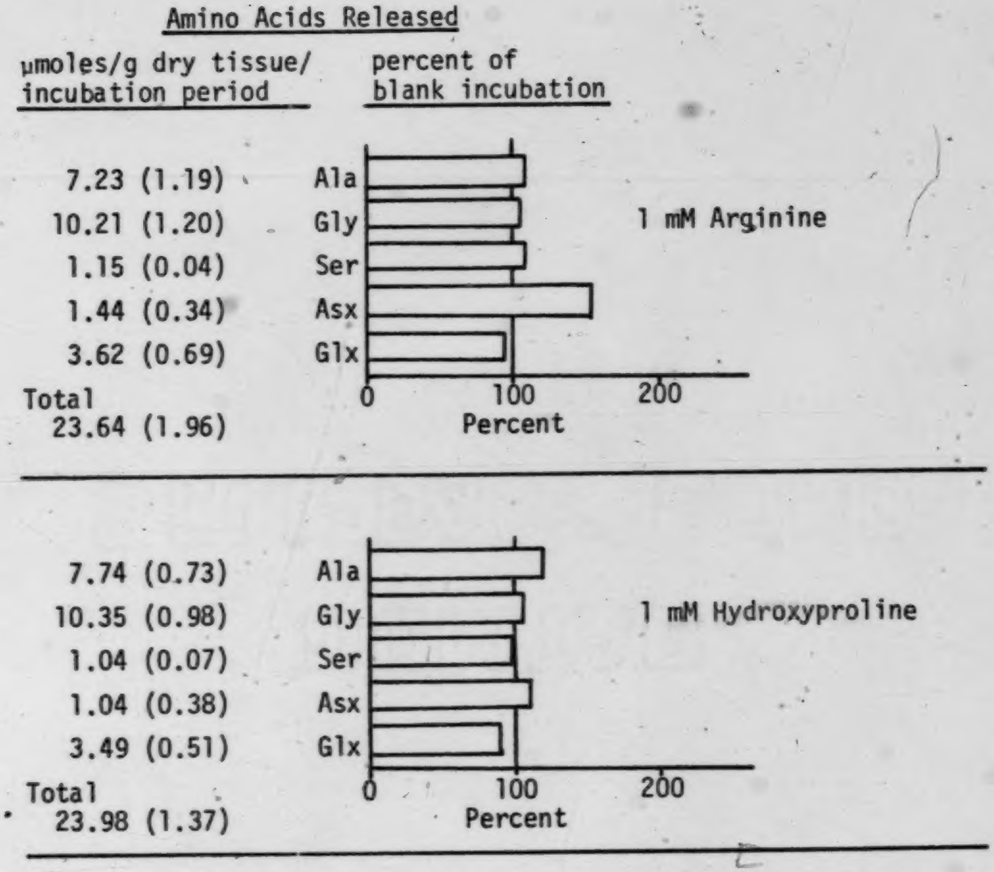
but none of the other amino acids were influenced (Fig. 23). Addition of alanine plus glyoxylate increased glycine and serine to 310% and 193% of the blank incubation respectively. The addition of 2 mM hydroxylamine alone did not change the quantity of amino acids in the incubation medium markedly, nor did its inclusion with alanine plus glyoxylate have any influence.

4.8 Series V. Individual Amino Acids

4.8.1 Arginine

The addition of 1 mM arginine to the incubation medium (Fig. 24) increased aspartate plus asparagine release by rumen papillae to 152% of the blank incubation. This aspartate plus asparagine represented 6.2% of total reported amino acids released by rumen papillae as compared to 4.4% for the blank incubation (Table 15).

Incubation of 1 mM arginine with rumen papillae resulted in the release of 19.46 ± 5.69 µmoles of ornithine per g dry papillae per incubation period. This ornithine output represented 35% of the arginine (1 mM) present at the start of the incubation period. Approximately 80% of the added arginine was accounted for as arginine plus ornithine in the incubation medium after 3 h of incubation. Citrulline may constitute a portion of the measured ornithine released by rumen papillae since it was not possible to separate the ornithine and citrulline esters using the GLC method described in Appendix B. In a mixture of amino acids of known concentration, the RMR obtained with ornithine was used to calculate the quantity of ornithine plus citrulline present in incubation media.



Amino Acids Released

µmoles/g dry tissue/ incubation period	
7.23 (1.19)	Ala
10.21 (1.20)	Gly
1.15 (0.04)	Ser
1.44 (0.34)	Asx
3.62 (0.69)	Glx
Total 23.64 (1.96)	

µmoles/g dry tissue/ incubation period	
7.74 (0.73)	Ala
10.35 (0.98)	Gly
1.04 (0.07)	Ser
1.04 (0.38)	Asx
3.49 (0.51)	Glx
Total 23.98 (1.37)	

Fig.24. Series V. Amino acid release by rumen papillae incubated with arginine or hydroxyproline. (Blank incubation Fig.20; mean followed by standard error in parentheses)

Table 15. Series V. Amino acids calculated as a percent of the total reported amino acids released by rumen papillae incubated with arginine or hydroxyproline.

Treatment	Amino Acid				
	Ala	Gly	Ser	Asx	Glx
Blank incubation	29.3 (2.3)[1]	43.7 (2.8)	4.8 (0.2)	4.4 (1.8)	17.8 (3.1)
NH_3-N (1 mM) + 0	35.0 (0.5)	42.7 (1.7)	4.3 (0.4)	3.6 (1.2)	14.3 (0.7)
Arginine (1 mM)	30.2 (3.3)	43.0 (2.5)	4.9 (0.4)	6.2 (1.7)	15.7 (3.7)
Hydroxyproline (1 mM)	32.2 (1.9)	43.0 (2.6)	4.4 (0.5)	4.4 (1.7)	14.8 (2.7)

1. Mean (n=3) followed by standard error in parentheses.

4.8.2 Hydroxyproline

The incubation of 1 mM hydroxyproline with rumen papillae (Fig. 24) did not change the pattern of amino acid release markedly when compared to the blank incubation. The release of amino acids by rumen papillae in the presence of added hydroxyproline were in the range of 89 to 117% of the blank incubation (Fig. 24).

4.9 Series VI Incubations

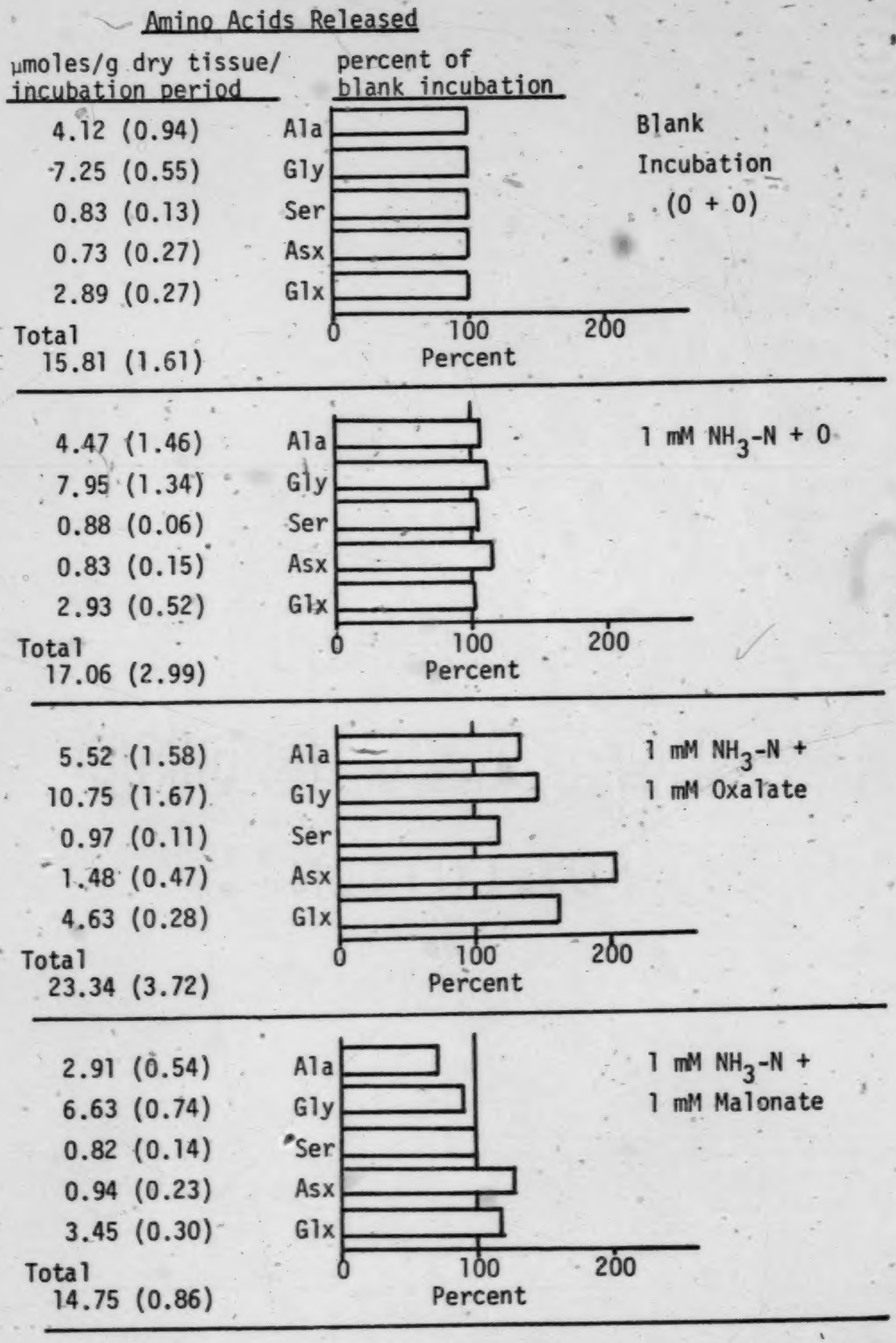
4.9.1 Oxalate and Malonate Incubations

Oxalate at 1 mM in the incubation medium increased the release of all reported amino acids from rumen papillae (Fig. 25). Total amino acids released were increased 48% when compared to the blank, or 37% when compared to NH_3-N incubated alone (NH_3-N + 0). In Series III, 10 mM oxalate did not influence alanine release by rumen papillae (Fig. 15). The percent of total reported amino acids in the presence of 1 mM oxalate was similar to the blank incubation for Series VI (Table 16).

With 1 mM malonate present in incubation media (Fig.25), the releases of alanine, aspartate plus asparagine and glutamate plus glutamine were 71%, 129% and 119% of the blank incubation respectively. Glycine and serine release did not change for the malonate incubations when compared to the blank incubation.

4.9.2 Ethanolamine

This incubation was included as an adjunct to Series III incubation, where glycine synthesis from two-carbon compounds was studied (Figs. 14 and 15). By incubating rumen papillae with 1 mM ethanolamine, alanine, glycine, serine and aspartate plus asparagine were released to 139%, 122%, 118% and



Treatment	Amino acid	µmoles/g dry tissue/ incubation period
Blank Incubation (0 + 0)	Ala	4.12 (0.94)
	Gly	7.25 (0.55)
	Ser	0.83 (0.13)
	Asx	0.73 (0.27)
	Glx	2.89 (0.27)
	Total	15.81 (1.61)
1 mM NH_3-N + 0	Ala	4.47 (1.46)
	Gly	7.95 (1.34)
	Ser	0.88 (0.06)
	Asx	0.83 (0.15)
	Glx	2.93 (0.52)
	Total	17.06 (2.99)
1 mM NH_3-N + 1 mM Oxalate	Ala	5.52 (1.58)
	Gly	10.75 (1.67)
	Ser	0.97 (0.11)
	Asx	1.48 (0.47)
	Glx	4.63 (0.28)
	Total	23.34 (3.72)
1 mM NH_3-N + 1 mM Malonate	Ala	2.91 (0.54)
	Gly	6.63 (0.74)
	Ser	0.82 (0.14)
	Asx	0.94 (0.23)
	Glx	3.45 (0.30)
	Total	14.75 (0.86)

Fig.25. Series VI. Amino acid release by rumen papillae incubated with ammonia nitrogen plus oxalate or malonate. (Mean followed by standard error in parentheses)

Table 16. Series VI. Amino acids calculated as a percent of the total reported amino acids released by rumen papillae incubated with: A. ammonia nitrogen plus oxalate or malonate; B. ethanolamine; and C. ammonia nitrogen plus acetate, propionate or butyrate.

Treatment	Amino Acid				
	Ala	Gly	Ser	Asx	Glx
Blank Incubation	25.3 (3.6)[1]	46.2 (2.2)	5.3 (0.8)	4.8 (1.8)	18.4 (0.7)
NH_3-N + 0[2]	25.2 (4.2)	46.8 (2.8)	5.5 (1.1)	5.2 (1.6)	17.2 (1.5)
A. NH_3-N + Oxalate (1 mM)	22.4 (4.0)	46.1 (0.5)	4.3 (0.3)	6.6 (1.9)	20.6 (2.4)
NH_3-N + Malonate (1 mM)	19.5 (2.6)	44.7 (3.4)	5.7 (1.2)	6.6 (2.0)	23.5 (2.0)
B. Ethanolamine (1 mM)	29.2 (3.6)	46.1 (2.6)	5.1 (0.8)	4.4 (1.2)	15.3 (1.2)
C. NH_3-N + Acetate (10 mM)	22.9 (5.5)	49.3 (2.7)	6.0 (1.2)	6.0 (1.6)	15.8 (1.8)
NH_3-N + Propionate (10 mM)	34.2 (6.6)	33.9 (0.6)	13.4 (1.8)	6.2 (1.6)	12.2 (3.0)
NH_3-N + Butyrate (10 mM)	32.0 (3.8)	41.6 (2.2)	5.5 (0.4)	4.8 (1.3)	16.1 (1.3)

1. Mean (n=3) followed by standard error in parentheses.
2. The concentration of NH_3-N for all treatments was 1 mM.

115% of the blank incubation respectively (Fig. 26); glycine release was not influenced selectively.

4.9.3. Glycine and One-Carbon Compounds

The addition of 1 mM formaldehyde and 1 mM formate separately to 1 mM glycine incubations increased the quantity of serine present in the incubation medium 27% and 71% respectively when compared to an addition of only glycine (Fig. 26). Serine was the only amino acid meaningfully influenced by formaldehyde or formate in the presence of glycine. Alanine recovery was 128%, 125% and 134% of the blank incubation with glycine, glycine plus formaldehyde and glycine plus formate incubations respectively (Fig. 26).

4.9.4. Incubations With Volatile Fatty Acids

The release of amino acids by rumen papillae with added acetate (Fig. 27) was in the range 86 to 111% of the blank incubation for all amino acids except aspartate plus asparagine which was 126% of the blank. With propionate as a substrate, alanine and serine were released to 121% and 204% of the blank incubation respectively, while glycine and glutamate plus glutamine release were 62% and 51% of the blank incubation respectively(Fig. 27). With butyrate as a substrate alanine was 126% of the blank incubation (Fig. 22); the release of other amino acids by rumen papillae in the presence of butyrate were in the range of 88 to 105% of the blank incubation (Fig. 27).

4.9.5 Amino Donors With Propionate

The inclusion of 1 mM glutamate in incubations of 1 mM NH_3-N plus 10 mM propionate (Fig. 28) increased the release of alanine by rumen

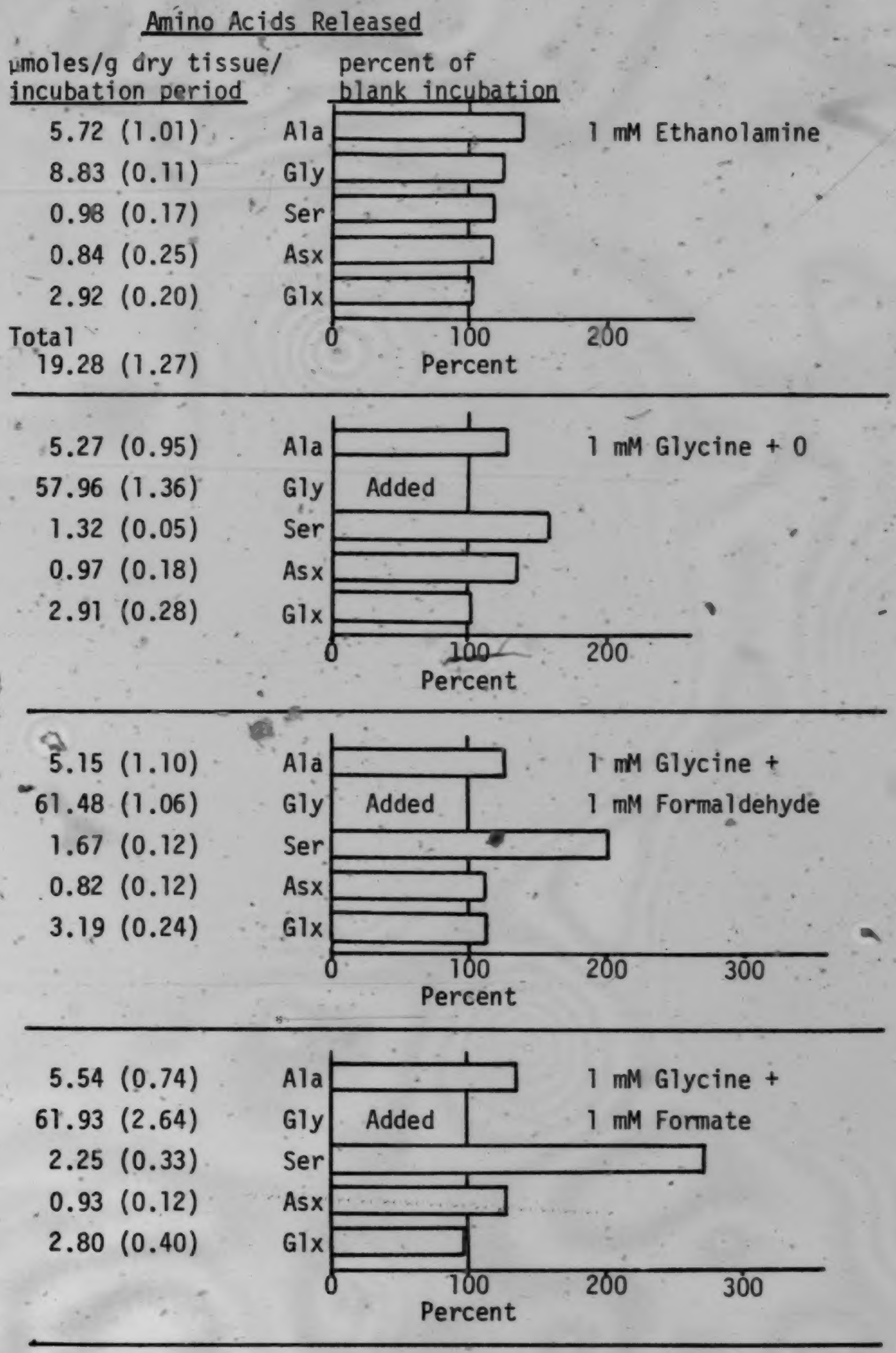


Fig.26. Series VI. Amino acid release by rumen papillae incubated with ethanolamine or glycine plus the one-carbon compounds formaldehyde or formate. (Blank incubation Fig.25; mean followed by standard error in parentheses)

Amino Acids Released

µmoles/g dry tissue/ incubation period	
3.81 (1.20)	Ala
7.71 (0.59)	Gly
0.92 (0.14)	Ser
0.92 (0.22)	Asx
2.48 (0.23)	Glx
Total 15.92 (1.62)	

µmoles/g dry tissue/ incubation period	
4.97 (1.86)	Ala
4.50 (0.97)	Gly
1.69 (0.19)	Ser
0.75 (0.15)	Asx
1.47 (0.10)	Glx
Total 13.37 (3.05)	

µmoles/g dry tissue/ incubation period	
5.20 (0.98)	Ala
6.56 (0.29)	Gly
0.87 (0.06)	Ser
0.77 (0.25)	Asx
2.53 (0.08)	Glx
Total 15.92 (1.42)	

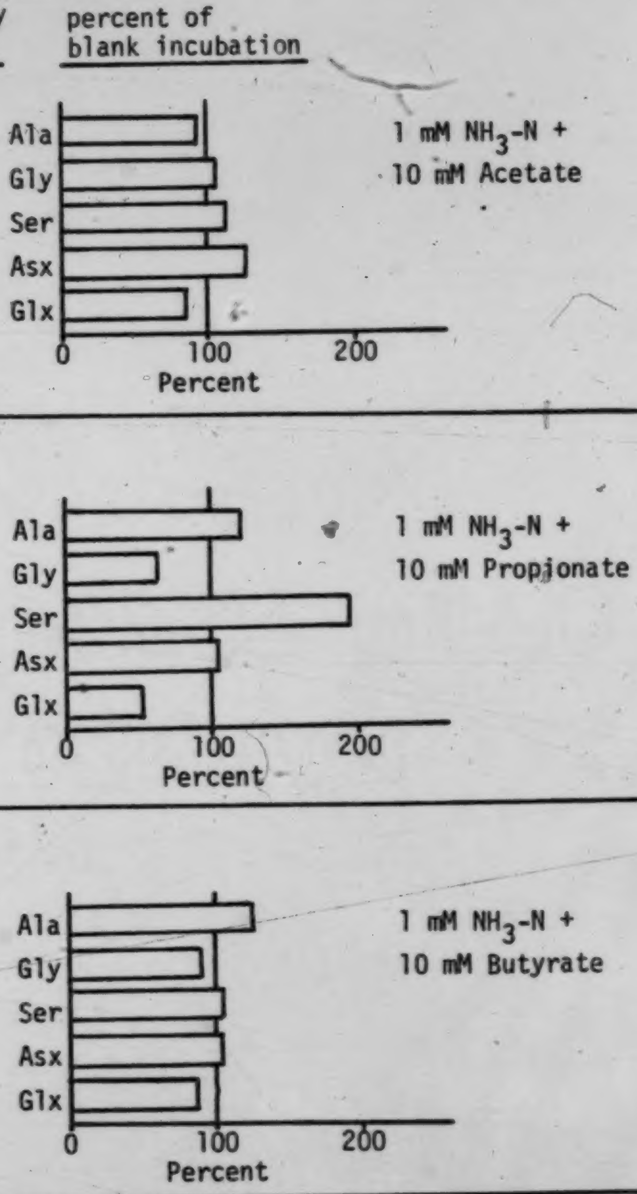


Fig.27. Series VI. Amino acid release by rumen papillae incubated with ammonia nitrogen plus acetate, propionate or butyrate. (Blank incubation Fig.25; mean followed by standard error in parentheses)

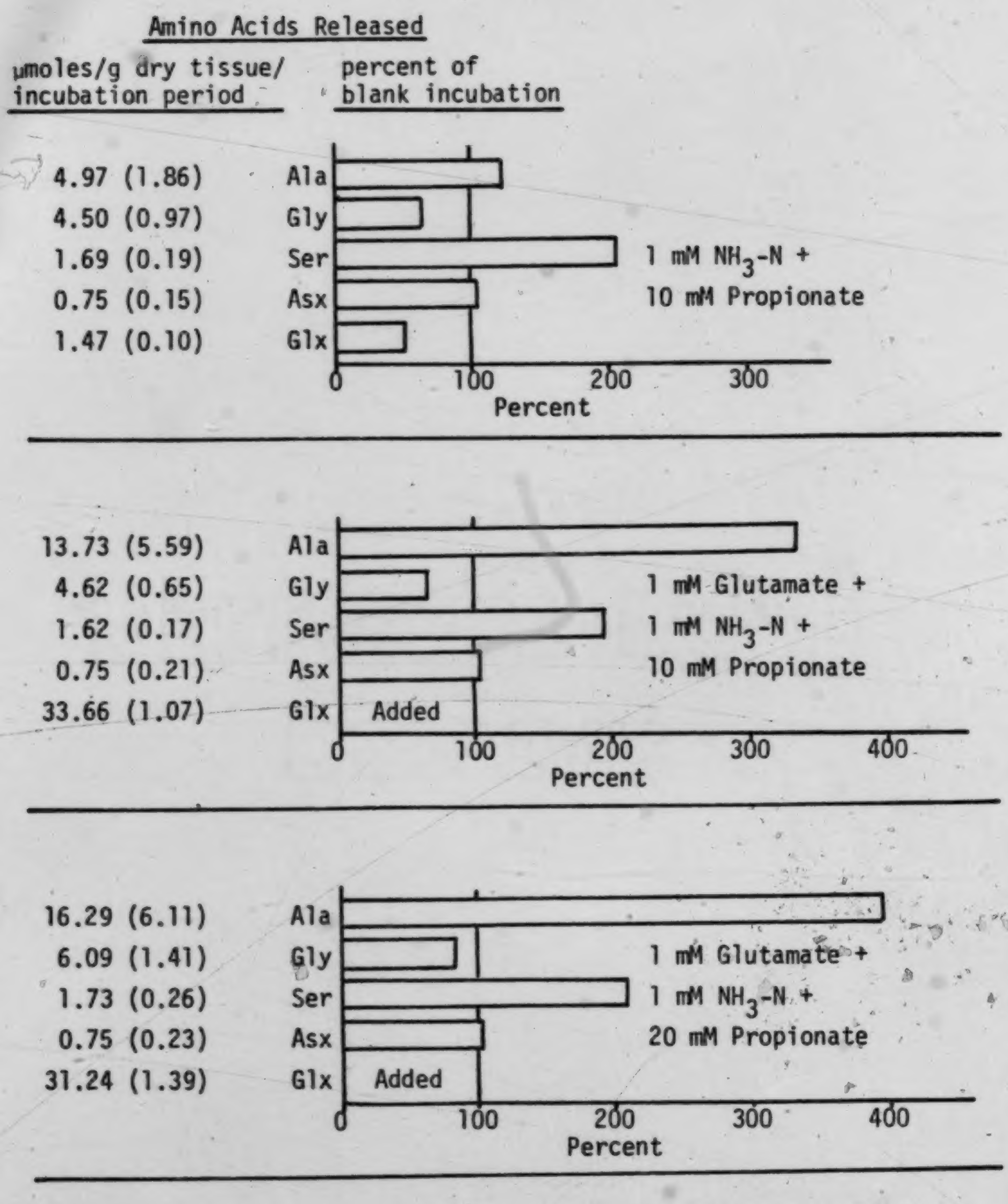


Fig.28. Series VI. Amino acid release by rumen papillae incubated with ammonia nitrogen plus 10 mM propionate, or glutamate plus ammonia nitrogen plus 10 or 20 mM propionate. (Blank incubation Fig.25; mean followed by standard error in parentheses)

papillae by 176% from 4.97 to 13.73 μmoles per g dry papillae per incubation period. Raising the propionate concentration to 20 mM with glutamate plus NH_3-N further increased alanine release to 16.29 μmoles per g dry papillae per incubation period. The quantity of serine in the incubation medium was not changed with the addition of glutamate in the presence of propionate or by an increased propionate concentration (Fig. 28).

4.9.6 Incubations for 1, 2 or 3 Hours

Two sets of incubations, including a blank incubation (no added nitrogen or carbon substrates) and a 1 mM glutamate plus 1 mM NH_3-N plus 10 mM propionate incubation were analyzed after 1, 2 and 3 h of incubation (Table 17). The rates of release of total reported amino acids for the blank incubation and the glutamate plus NH_3-N plus propionate incubation were quite similar (Fig. 29).

4.9.6.1 The Blank Incubation

The rate of alanine release by rumen papillae was greatest during the first hour; the total alanine released did not change markedly thereafter (Fig. 30). Glycine release was nearly linear up to 2 h and increased only slightly from 2 to 3 h of incubation. Glutamate plus glutamine release increased at a lesser rate after 1 h of incubation. The release of serine or aspartate plus asparagine was low and did not change after 2 h of incubation. With no substrates added, the quantities of amino acid released to the incubation medium were in the order glycine > alanine > glutamate plus glutamine > serine = aspartate plus asparagine, and in fact the only notable release after 1 h was for glycine.

Table 17. Series VI. Amino acid release by rumen papillae after 1, 2 and 3 hours of incubation for the blank incubation and the 1 mM glutamate plus 1 mM ammonia nitrogen plus 10 mM propionate incubation.

Length of Incubation (Hours)	Amino Acid[1]					Total
	Ala	Gly	Ser	Asx	Glx	
Blank Incubation						
1	3.63 (0.32)[2]	3.99 (0.13)	0.58 (0.08)	0.49 (0.20)	2.00 (0.23)	10.69 (0.29)
2	4.99 (0.35)	6.95 (0.43)	0.84 (0.08)	0.75 (0.07)	2.22 (0.06)	15.76 (0.67)
3	4.12 (0.94)	7.25 (0.55)	0.83 (0.13)	0.73 (0.27)	2.89 (0.27)	15.81 (1.61)
1 mM Glutamate + 1 mM NH_3-N + 10 mM Propionate						
1	7.38 (1.65)	4.14 (0.48)	0.88 (0.18)	0.57 (0.11)	37.61 (3.28)	12.97 (1.34)[3]
2	9.99 (3.00)	4.89 (0.47)	1.34 (0.09)	0.75 (0.15)	35.69 (0.29)	16.97 (3.04)
3	13.73 (5.59)	4.62 (0.65)	1.62 (0.17)	0.75 (0.21)	33.66 (1.07)	20.72 (6.28)

1. µmoles of amino acid per gram dry rumen papillae per incubation period.
2. Mean (n=3) followed by standard error in parentheses.
3. Since glutamate was added to the glutamate plus NH_3-N plus propionate incubations the total was calculated minus Glx.

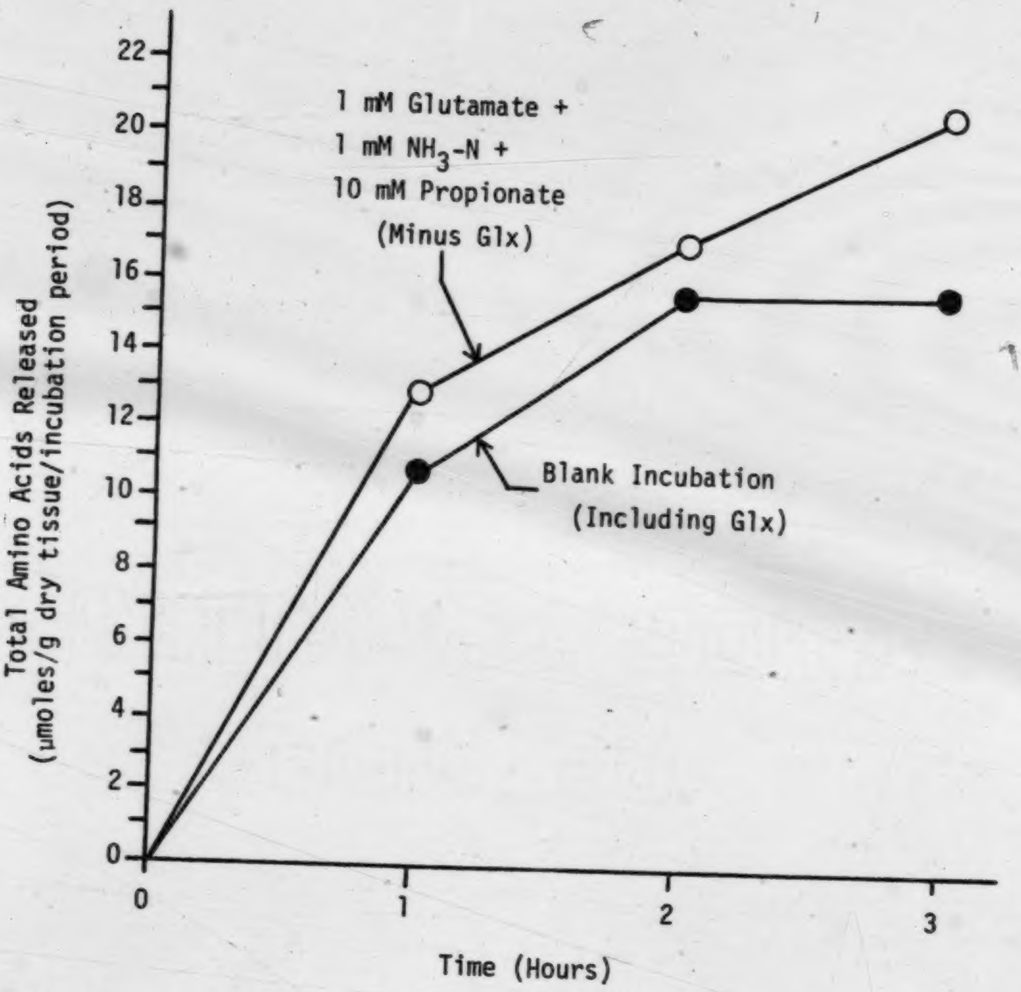


Fig.29. Series VI.The release of total reported amino acids by rumen papillae after 1, 2 and 3 hours of incubation for the blank incubation and the 1 mM glutamate plus 1 mM ammonia nitrogen plus 10 mM propionate incubation. (Refer to Table 17 for an explanation of including and minus Glx)

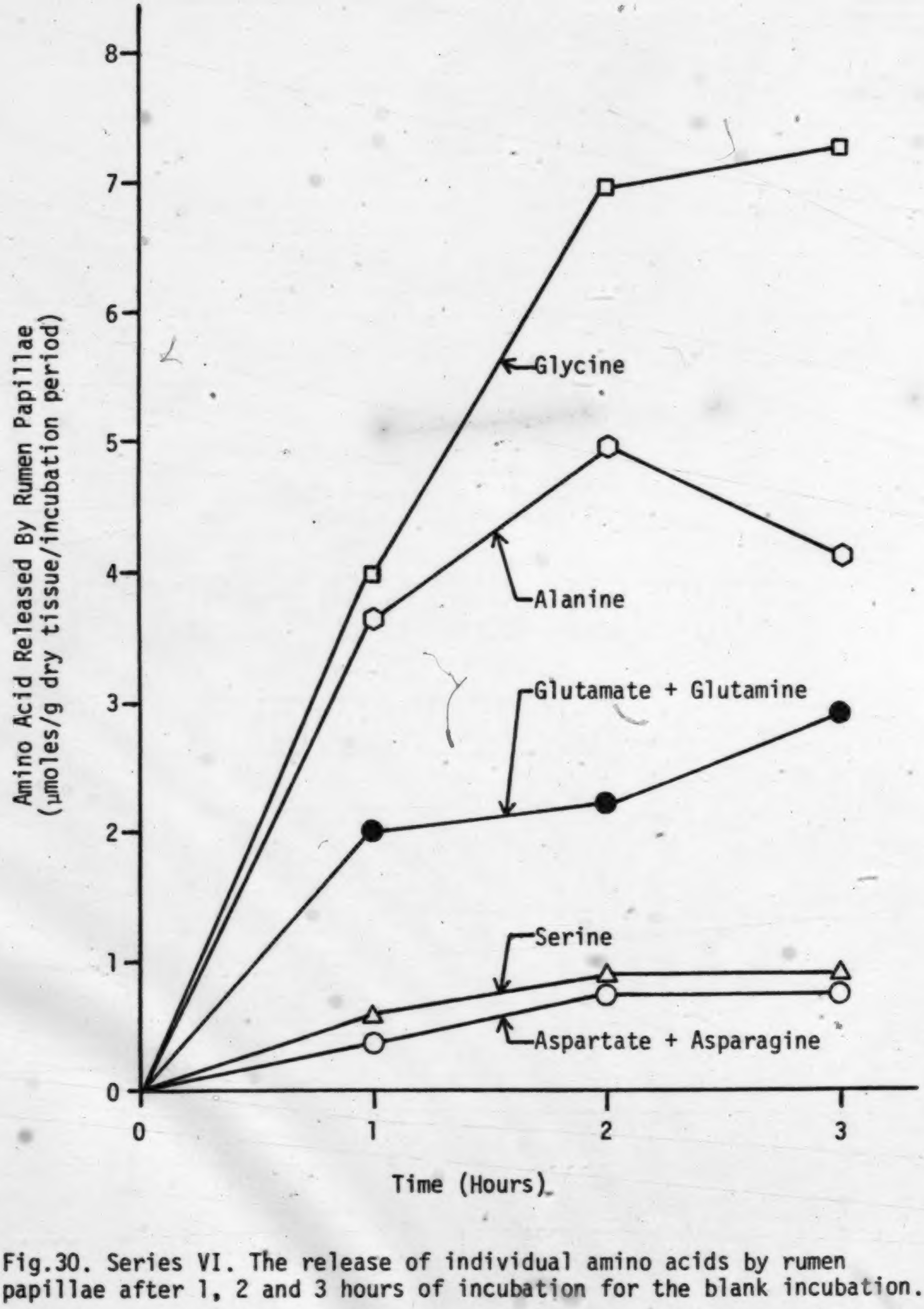


Fig.30. Series VI. The release of individual amino acids by rumen papillae after 1, 2 and 3 hours of incubation for the blank incubation.

4.9.6.2. The Glutamate Plus Ammonia Nitrogen Plus Propionate Incubation

The incubation of rumen papillae with glutamate plus NH_3-N plus propionate (Fig. 31) resulted in a very different pattern of amino acid release than the blank incubation (Fig. 30). Alanine production (Fig. 31) was rapid over the 3 h period, while glycine release did not change after 1 h of incubation. Serine release was low and relatively constant, while aspartate plus asparagine appeared to increase to 2 h and remained constant thereafter. Amounts of amino acids released into the incubation medium with glutamate plus NH_3-N plus propionate as substrates were in the order alanine > glycine > serine = aspartate plus asparagine.

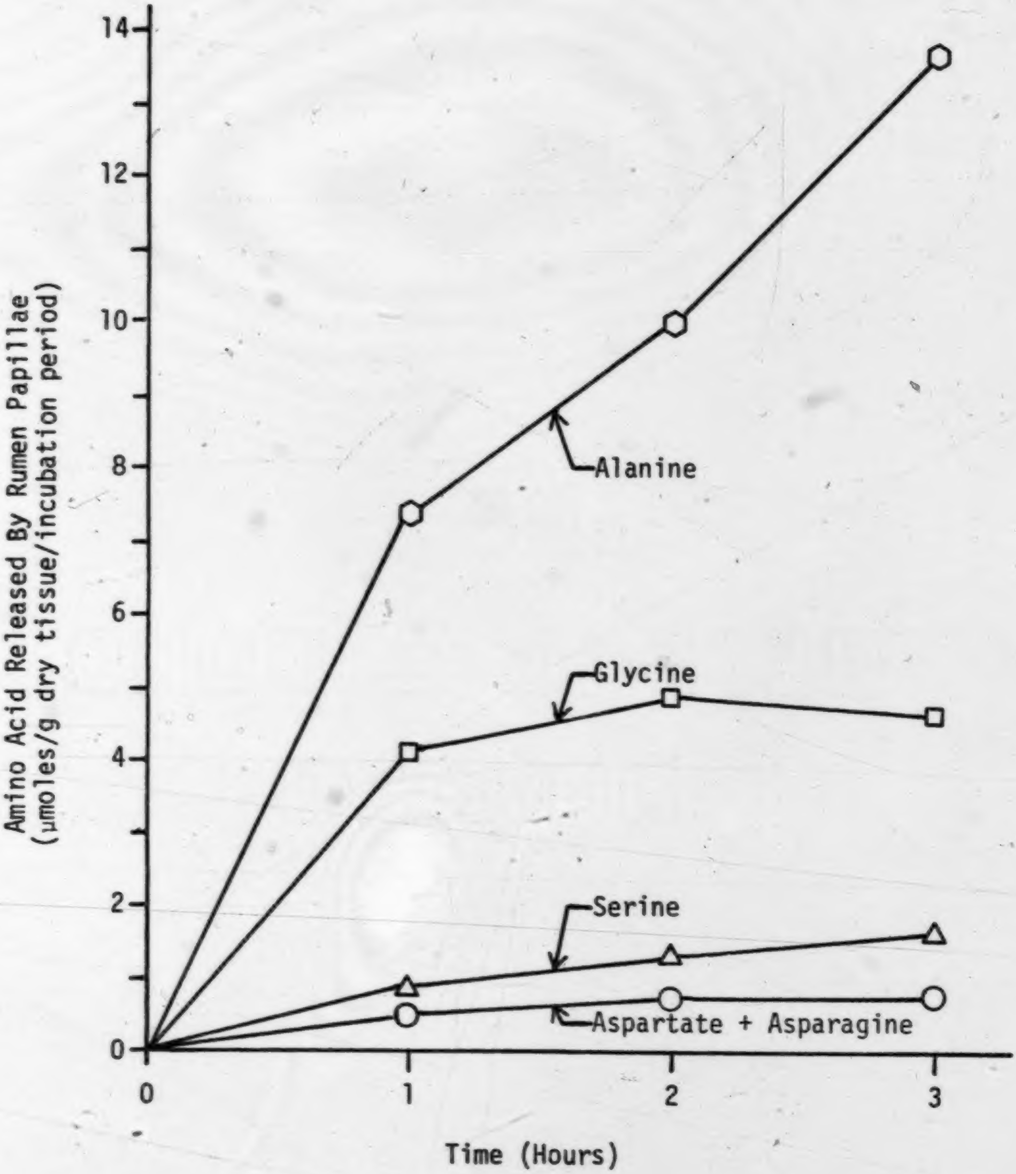


Fig.31. Series VI. The release of individual amino acids by rumen papillae after 1, 2 and 3 hours of incubation for the 1 mM glutamate plus 1 mM ammonia nitrogen plus 10 mM propionate incubation.

5. DISCUSSION

5.1 In Vitro Preparations

The metabolism of whole rumen papillae was examined in this study. Rumen papillae consist of a keratinized stratified squamous epithelium, covering a central core of densely packed collagen fibres with no associated muscle fibres (Dobson et al. 1956). Radiating from this central core is a highly branched system of blood and lymphatic vessels. Numerous and densely packed mitochondria are associated with the columnar cells of the metabolically active stratum basale of the epithelium (Dobson et al. 1956).

Epithelium from rumen tissue kept at 39 C in Krebs-Ringer Phosphate minus calcium for 20 min after removal from the animal was found by Hodson et al. (1967) to metabolize butyrate more rapidly to ketone bodies than tissue kept on ice for 20 min. Tissue kept at 0 C before incubation contained distorted and ruptured mitochondria with an apparent lack of cristae. Therefore, in the present study, rumen tissue was transported at 39 C in the buffers used by Hodson et al. (1967) in an effort to retain functional mitochondria.

A 10 mM concentration of an individual organic acid present in incubation media served as the primary carbon source for metabolism by rumen papillae. Since substrates were present at concentrations manifold greater than physiological concentrations (Prior et al. 1972), the activity of reactions other than those under investigation may have been changed. Consideration of the effect of an added carbon substrate was possible on the basis of documented information of amino acid metabolism in tissues such as liver and kidney (Sallach and Fahien 1969; Greenberg 1969a, 1969b), the enzyme profiles of rumen tissue (Whanger and Church 1970), and the possible metabolic pathways available in rumen tissue (Pennington and Sutherland 1956a, 1956b; Pennington and Pfander 1957).

The amino acids released by rumen papillae in response to the addition of organic acids, amino acids and NH_3-N were alanine, glycine, serine, aspartate plus asparagine and glutamate plus glutamine. The quantities of amino acids released by rumen papillae were in the order of glycine > alanine > glutamate plus glutamine > serine = aspartate plus asparagine for incubations without added substrates (Fig. 30); this relationship changed when substrates were added to incubation media (compare Fig. 31 with Fig. 30). Ornithine plus citrulline release by rumen papillae was detected only upon inclusion of arginine in incubation media.

During preparation of amino acid esters for GLC analysis, glutamine was converted to glutamate and asparagine to aspartate, while during GLC analysis, the esterification products of citrulline and ornithine had the same retention characteristics (Appendix B). The contribution of glutamine to a sum of glutamate plus glutamine is discussed in the section entitled "Glutamine Metabolism", while the differentiation of ornithine from citrulline release by rumen papillae is discussed in the section entitled "Arginine Metabolism".

5.2 The Synthesis of Amino Acids from Carbon Sources

Incubation of a 10 mM carbon source with rumen papillae always increased the release of one or more amino acids (Table 18) relative to the

Table 18. Amino acids influenced to the largest extent by the addition of carbon sources.

Carbon Source	Series	Amino acids released by rumen papillae in quantities greater than the blank incubation
Propionate	I,VI	Alanine,[1] Serine
Succinate	I	Serine
Malate	I	Aspartate plus Asparagine
Pyruvate	I	Alanine, Serine
Glyoxylate	I,III	Glycine, Serine
α-Ketoglutarate	I,III	Glutamate plus Glutamine
Citrate	III	Glutamate plus Glutamine
Glucose	III	Serine, Alanine[2]

1. A demonstration of alanine release by rumen papillae incubated with propionate required glutamate as a nitrogen source (Fig.13).
2. Although glucose resulted in a decrease in total amino acids released by rumen papillae (Fig.17), the relative quantity of alanine increased (Table 12), but the total alanine released by rumen papillae was less than the blank incubation.

blank incubation. It would be desirable to know whether or not there was utilization of the added carbon sources for synthesis of amino acids, or if added substrates simply had secondary effects on the release of endogenous amino acids by rumen papillae.

From the data reported by Salem et al.(1973b), it was calculated, assuming 18% dry matter for rumen epithelium (Appendix Table 1), that the concentrations of alanine, glycine, aspartate and glutamate, as free amino acids in rumen epithelium were 2.5, 4.8, 1.9 and 6.6 μmoles per gram dry tissue respectively. The release of amino acids by rumen papillae in the blank incubations for Series I to VI was for alanine, glycine, aspartate plus asparagine and glutamate plus glutamine in the range of 4.0 to 10.5, 7.3 to 11.5, 0.7 to 1.0 and 3.0 to 5.0 μmoles per gram dry rumen papillae per incubation period respectively. This would indicate that the amino acids released by rumen papillae in the blank incubation might be attributed to the free amino acids present in rumen papillae.

As a result of inclusion of carbon sources in incubation media, rumen papillae released amino acids in both lesser (40 to 60% of the blank) and greater (150 to 200% of the blank and higher) quantities than the blank incubation. The amounts of amino acids released in the presence of carbon sources usually exceeded the calculated amounts of free amino acids in rumen papillae. Enhanced proteolysis in rumen papillae upon addition of substrates to incubation media was not indicated since the increased amino acid release was usually the expected product, rather than an overall release of amino acids by rumen papillae. Therefore in most cases with the addition of carbon substrates, the increased release of amino acids reflected synthesis of amino acids from the particular carbon substrate.

The synthesis of alanine, glycine, glutamate, serine and aspartate are discussed in separate sections.

Lack of an effect of added substrates (e.g. glyoxal, Fig. 14; glycollate, Fig. 15; hydroxyproline, Fig. 24) on amino acid release by rumen papillae might have resulted from an inability to metabolize the substrates, or might have been due to the existence of permeability barriers in the tissues of rumen papillae. Weekes (1974) listed these permeability barriers in intact rumen papillae to be the outer layers of the stratum corneum, the cell membranes of the metabolically active cells of the stratum basale and the mitochondrial membranes of these cells.

5.2.1 The Synthesis of Alanine

Pyruvate incubated with NH_3-N (Fig. 8) or with glutamate (Fig. 12) as the nitrogen source increased the release of alanine by rumen papillae when compared to a blank incubation, while propionate increased the release of alanine only in the presence of glutamate as an amino donor (Fig. 13). The release of alanine with glucose as a substrate (Table 18) was best demonstrated with alanine calculated as a percent of total reported amino acids (Table 12; compare alanine for the glucose and blank incubations). Alanine is synthesized by transamination of pyruvate in liver tissue (Sallach and Fahien 1969); the detection of alanine aminotransferase in rumen tissue (Whanger and Church 1970; Weekes 1972) confirmed that rumen papillae have the potential to synthesize alanine from pyruvate (Fig. 32). Weekes (1974) demonstrated in vitro that both propionate and glucose are sources for the production of pyruvate by rumen papillae.

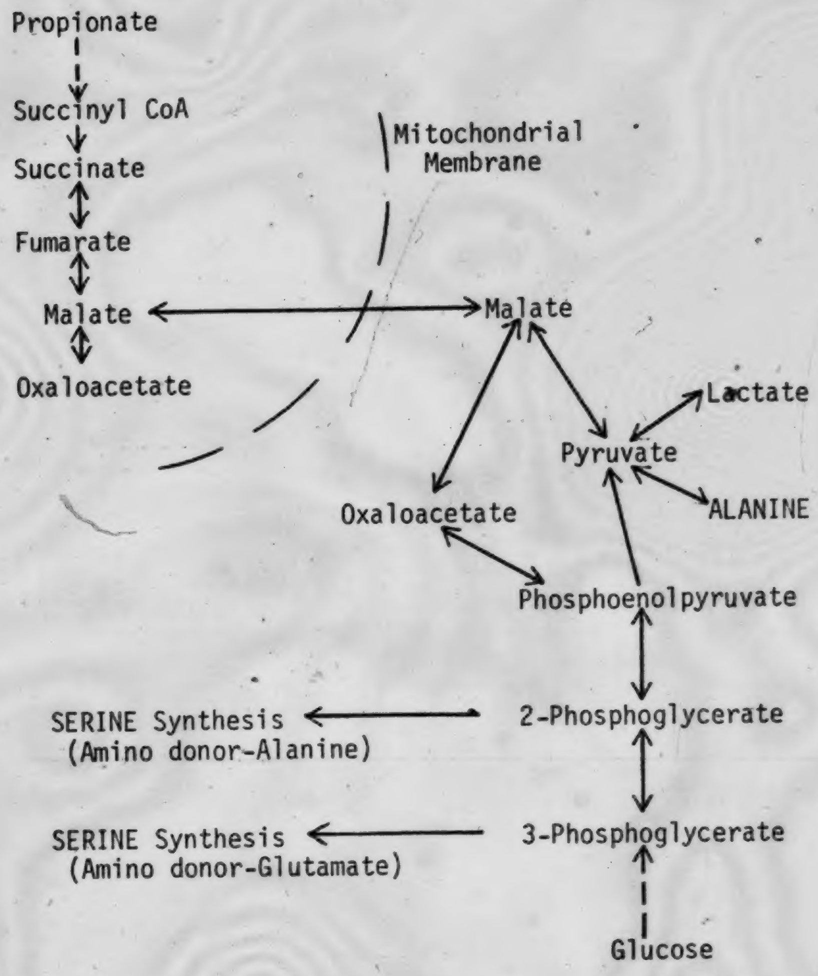

Propionate
Succinyl CoA
Succinate
Fumarate
Malate
Oxaloacetate
Mitochondrial Membrane
Malate
Lactate
Pyruvate
ALANINE
Oxaloacetate
Phosphoenolpyruvate
SERINE Synthesis (Amino donor-Alanine)
2-Phosphoglycerate
SERINE Synthesis (Amino donor-Glutamate)
3-Phosphoglycerate
Glucose

Fig.32. The synthesis of alanine and serine by rumen papillae from propionate, succinate, pyruvate and glucose as carbon sources. (Leng et al. 1967; Pennington and Sutherland 1956b; Pennington and Pfander 1957; Greenberg 1969b; Young et al. 1969; Whanger and Church 1970; Weekes 1972,1974)

Lactate (Fig. 7), unlike propionate (Fig. 8) which may be its precursor in vitro (Pennington and Sutherland 1956b), did not modify the release of alanine or serine by rumen papillae when compared to an incubation with no added substrates. Jones et al.(1969) recovered [^{14}C] in alanine when ground rumen tissue was incubated with 25, 75 or 125 mM [U-^{14}C] lactate. This transfer of label probably results from a synthesis of alanine from pyruvate that had been equilibrated with lactate by lactate dehydrogenase (EC 1.1.1.28) and likely does not represent a net synthesis of alanine by rumen tissue with lactate as a substrate.

5.2.2. The Synthesis of Glycine

Rumen papillae synthesized glycine using glyoxylate as a carbon source (Figs. 8 and 15). The two-carbon compounds, glycolaldehyde, glyoxal and glycollate are metabolized to glyoxylate in rat liver (Weinhouse and Friedman 1951; Weisbach and Sprinson 1953a; Arnstein 1954; Fig. 3), but did not increase the release of glycine by rumen papillae (Figs. 14 and 15). Either the pathways necessary for the conversion of glycolaldehyde, glyoxal or glycollate to glyoxylate are not present in rumen papillae or these substrates never reached the enzyme sites for metabolism to glyoxylate.

Ethanolamine, also a two-carbon compound, can be converted to glycine either via choline, betaine and sarcosine, or via glycolaldehyde (Fig. 3) according to a scheme presented by Meister (1965). Since ethanolamine increased the release of glycine (Fig. 26) in the present study, but glycolaldehyde did not (Fig. 14), the former pathway for ethanolamine may have been operative.

Adams (1971) and Hagler and Herman (1973) reported that hydroxyproline is converted to glyoxylate in liver tissue. However, inclusion of 1 mM hydroxyproline (Fig. 24) did not change amino acid release by rumen papillae when compared to its blank incubation in this study. The impermeability of the tissues to hydroxyproline, the absence of enzymes that metabolize hydroxyproline to glyoxylate, or the low 1 mM concentration used in this study might have prevented hydroxyproline from having an influence upon amino acid release by rumen papillae.

5.2.3 The Synthesis of Glutamate

It has been reported that glutamate dehydrogenase is present in rumen papillae (Hoshino et al. 1966; Chalupa et al. 1970; Whanger and Church 1970). When rumen papillae were incubated with 10 mM α-ketoglutarate, there was an increased release of glutamate plus glutamine (Figs. 9 and 17). This would suggest that glutamate, or glutamine formed from glutamate may be synthesized in this tissue, and serve as an amino donor for the production of alanine, glycine and aspartate from pyruvate, glyoxylate and oxaloacetate respectively.

Citrate increased the recovery of glutamate plus glutamine to 164% of the blank incubation (Fig. 17). A functional tricarboxylic acid cycle was reported to be present in rumen mucosa (Pennington and Sutherland 1956a; Seto et al. 1970). Therefore conversion of citrate to α-ketoglutarate, and subsequent utilization of this α-ketoglutarate as a substrate for glutamate dehydrogenase could account for the increased recovery of glutamate plus glutamine with citrate as a carbon source.

5.2.4 The Synthesis of Serine

Incubation of rumen papillae with propionate, succinate, pyruvate and glucose increased the release of serine when compared to blank incubations (Table 18). In liver, kidney and brain tissue, the synthesis of serine from carbon sources involves the intermediates of glycolysis, 2-phosphoglycerate or 3-phosphoglycerate (Fig. 2; Greenberg 1969b).

Glucose increased the release of serine by rumen papillae to 155% of the blank incubation (Fig. 17). A glycolytic scheme in rumen papillae was confirmed when Pennington and Pfander (1957) and Weekes (1974) incubated rumen tissue with glucose in vitro and increased the production of pyruvate and lactate. With the presence of a pathway of glycolysis in rumen mucosa, one may anticipate that 2-phosphoglycerate and 3-phosphoglycerate are available as potential substrates leading to a synthesis of serine (Fig. 32).

In order for propionate, succinate and pyruvate to provide carbon for the synthesis of serine the carbon of these three compounds must be able to be metabolized to an intermediate of glycolysis, phosphoenolpyruvate and subsequently to 2-phosphoglycerate or 3-phosphoglycerate (Fig. 32). Propionate is known to be metabolized in vitro to lactate by rumen papillae (Pennington and Sutherland 1956b; Weekes 1974); Pennington and Sutherland (1956b) and Leng et al.(1967) reported that succinate, malate and pyruvate are intermediates for this conversion (Fig. 32). Malate can be converted to oxaloacetate by a reaction catalyzed by NAD malate dehyrodgenase (EC 1.1.1.37), and oxaloacetate to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (EC 6.4.1.1) (Fig. 32). The presence of the enzymes, NAD malate dehydrogenase and phosphoenolpyruvate carboxykinase in rumen mucosa was established by Whanger and Church (1970) and Young et al.(1969)

respectively. Although malate appears to be an intermediate for the conversion of propionate to serine, malate added to incubation media as a carbon source did not increase the release of serine by rumen papillae when compared to the blank incubation (Fig. 9). It was difficult to determine the basis for this observation with malate as a carbon source.

Glyoxylate, besides increasing the release of glycine by rumen papillae, also increased the release of serine (Figs. 8 and 15). A reaction catalyzed by serine hydroxymethyltransferase, could be responsible for the production of serine from glycine synthesized from glyoxylate.

5.2.5 The Synthesis of Aspartate

Addition of malate as a carbon source increased the release of aspartate plus asparagine by rumen papillae to 185% of the blank incubation (Fig. 9). It has been demonstrated that malate is able to be metabolized to oxaloacetate by NAD malate dehydrogenase. Whanger and Church (1970) reported the presence of an active NAD malate dehydrogenase and aspartate aminotransferase in rumen mucosa. Therefore, the carbon of malate was able to be converted to aspartate (plus asparagine) via oxaloacetate (Fig. 33).

5.2.6 Concluding Remarks - Utilization of Carbon Sources

Rumen papillae (most likely the ruminal epithelium) were able to synthesize amino acids from various carbon inputs (Table 18; Fig. 33). Due to its high concentration in fluid bathing the papillae (Rumsey et al. 1970), propionate may be the most available source of carbon for the synthesis of amino acids by rumen papillae.

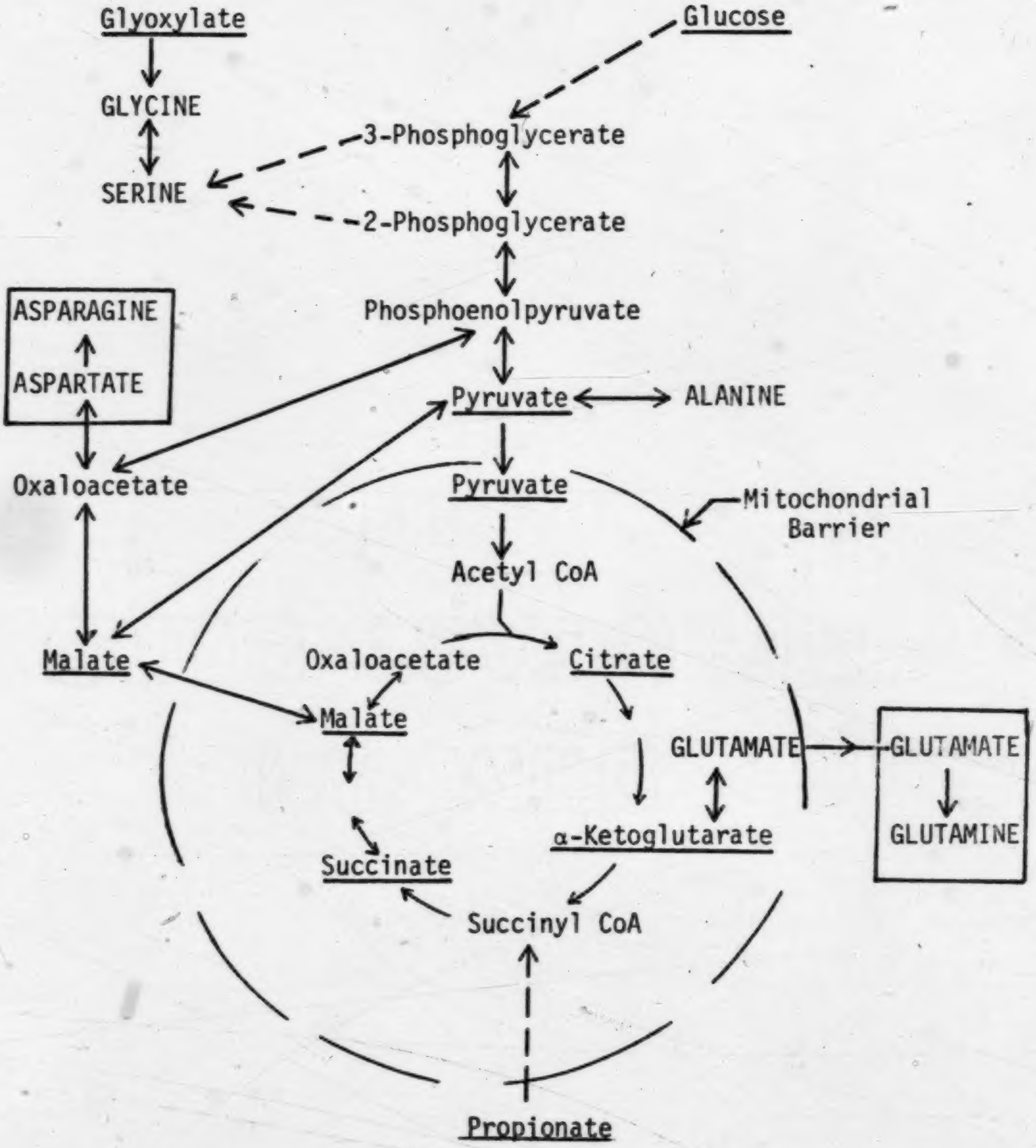

Glyoxylate
GLYCINE
SERINE
Glucose
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
ASPARAGINE
ASPARTATE
Oxaloacetate
Pyruvate
ALANINE
Pyruvate
Mitochondrial Barrier
Acetyl CoA
Malate
Oxaloacetate
Citrate
Malate
GLUTAMATE
GLUTAMATE
GLUTAMINE
α-Ketoglutarate
Succinate
Succinyl CoA
Propionate

Fig.33. Likely pathways of synthesis of amino acids in rumen papillae. (Components underlined were those added as carbon sources to incubation media)

5.3 Amino Donors

The treatments of Series II and Series V were designed to determine how effective glutamate, glutamine or alanine were as amino donors for the synthesis of amino acids by rumen papillae. For aminotransferase reactions leading to the synthesis of alanine, glycine or serine in liver, kidney, heart muscle or brain tissue, the most active amino donors are glutamate and glutamine (Sallach and Fahien 1969; Greenberg 1969b; Cooper and Meister 1974). Alanine also serves as an amino donor for the synthesis of glycine in human liver (Thompson and Richardson 1967) and the synthesis of serine in liver and kidney (Greenberg 1969b).

5.3.1 Glutamate and Glutamine as Amino Donors with Glyoxylate and Pyruvate

In the present study, glutamate appeared to be a more effective amino donor than glutamine for the synthesis of glycine (Fig. 11) and alanine (Fig. 12) from glyoxylate and pyruvate respectively. The glutamine treatments, when compared to treatments with glutamate, with or without NH_3-N, did not result in the additional release of glycine by rumen papillae in the presence of glyoxylate (Fig. 11) or the release of alanine in the presence of pyruvate (Fig. 12). In fact, glycine release by rumen papillae with 1 mM glutamine plus 10 mM glyoxylate (Fig. 11) and 1 mM NH_3-N plus 10 mM glyoxylate (Fig. 8) was similar; alanine release by rumen papillae with 1 mM glutamine plus 10 mM pyruvate (Fig. 12) and 1 mM NH_3-N plus 10 mM pyruvate (Fig. 8) was similar. Glutamine did not function as a direct amino donor in rumen papillae.

The enzymatic potential for the tissues of rumen papillae (~~ruminal~~ epithelium) to synthesize glutamate appears limited. Whanger and

Church (1970) reported that glutamate dehydrogenase activity per unit extractable protein from cattle rumen epithelium was only 10 to 15% of that in cattle liver. The release of glycine by rumen papillae with 1 mM α-ketoglutarate plus 1 mM NH_3-N plus 10 mM glyoxylate (Fig. 11) and 1 mM NH_3-N plus 10 mM glyoxylate (Fig. 8) was similar; the release of alanine by rumen papillae with 1 mM α-ketoglutarate plus 1 mM NH_3-N plus 10 mM pyruvate (Fig. 12) and 1 mM NH_3-N plus 10 mM pyruvate (Fig. 8) was similar. The inclusion of 1 mM α-ketoglutarate with 1 mM NH_3-N plus 10 mM glyoxylate (Fig. 11) or 10 mM pyruvate (Fig. 12), when compared to incubations of 1 mM NH_3-N plus 10 mM glyoxylate (Fig. 8) or 10 mM pyruvate (Fig. 8) respectively increased the release of glutamate plus glutamine but this glutamate plus glutamine was not able to sustain glycine synthesis from glyoxylate, or alanine synthesis from pyruvate in a manner approaching an inclusion of 1 mM glutamate (Figs. 11 and 12 for glyoxylate and pyruvate respectively). Glutamate from sources other than synthesized from α-ketoglutarate by rumen papillae (glutamate added to incubation media in this study) may contribute as a supplementary source of amino groups for the synthesis of amino acids by rumen papillae in vivo.

5.3.2 Glutamate for the Synthesis of Aspartate

The amounts of aspartate plus asparagine in incubation media were always increased with the addition of glutamate, with or without added NH_3-N, as compared to α-ketoglutarate plus NH_3-N or Glutamine when glyoxylate, pyruvate or propionate were the carbon sources (Figs 11, 12 and 13 respectively). An active aspartate aminotransferase, as reported by Whanger and Church (1970) in cattle ruminal epithelium, would likely account for the synthesis of aspartate (plus asparagine) from oxaloacetate. The α-ketoglutarate produced from the transamination reaction between glutamate and oxaloacetate to yield aspartate, would be expected to be converted to oxaloacetate through reactions of the tricarboxylic acid cycle. Consequently, a source of oxaloacetate would always be available for the synthesis of aspartate in the presence of added glutamate.

5.3.3 Alanine as an Amino Donor with Glyoxylate

The release of glycine by rumen papillae upon the inclusion of 1 mM alanine with 10 mM glyoxylate (Fig. 10 and 23) indicated that alanine-glyoxylate aminotransferase is present in rumen papillae. However it was found that hydroxylamine (Fig. 23), which has been reported to be an inhibitor of alanine-glyoxylate aminotransferase (Thompson and Richardson 1967) did not have an effect on glycine release in the presence of alanine and glyoxylate. This lack of effect could have been due to a variety of reasons including resistance to inhibition on the part of the enzyme in rumen papillae, an inability of hydroxylamine to reach the site of the enzyme because of permeability barriers, or perhaps an absence of alanine-glyoxylate aminotransferase with the formation of glycine by an alternate route in the presence of added alanine.

A combination of pathways (Reaction 1 to 4, Fig. 34) might account for the additional glycine released by rumen papillae upon the inclusion of 1 mM alanine (Figs. 10 and 23) as compared to 1 mM glutamate (Figs. 11 and 22) with 10 mM glyoxylate if no alanine glyoxylate aminotransferase was present. Synthesis of glutamate by transamination between alanine and α-ketoglutarate (Reaction 1, Fig. 34) followed by the use of this glutamate as an amino donor (Reaction 2, Fig. 34) might account for most

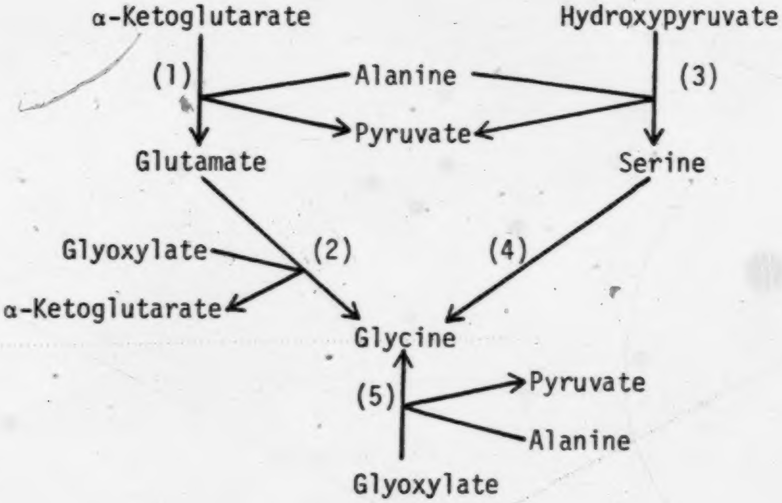

α-Ketoglutarate
Hydroxypyruvate
(1)
Alanine
(3)
Pyruvate
Glutamate
Serine
Glyoxylate
(2)
(4)
α-Ketoglutarate
Glycine
(5)
Pyruvate
Alanine
Glyoxylate

Fig.34. A series of reactions that may account for the synthesis of glycine from glyoxylate as a carbon source and alanine as a nitrogen source. (Thompson and Richardson 1966,1967; Greenberg 1969b)

Reaction
(1) Alanine aminotransferase (EC 2.6.1.2)
(2) Glycine aminotransferase (EC 2.6.1.4)
(3) Serine-pyruvate aminotransferase (EC 2.6.1.51)
(4) Serine hydroxymethyltransferase (EC 2.1.2.1)
(5) Alanine-glyoxylate aminotransferase (EC 2.6.1.44)

of the glycine present in the incubation medium. It was seen that glycine release in the presence of glyoxylate was enhanced upon the addition of glutamate (Fig. 11). Alanine has been reported to serve as an amino donor for the synthesis of serine from hydroxypyruvate in liver tissue (Greenberg 1969b; Reaction 3, Fig. 34). Conversion of serine to glycine by a reaction catalyzed by serine hydroxymethyltransferase (Reaction 4, Fig. 34) might then account for the extra glycine released by rumen papillae with 10 mM glyoxylate as the carbon source, and alanine (Figs. 10 and 23) as compared to glutamate (Figs. 11 and 22) as a nitrogen source. Glutamate plus glutamine did not accumulate in quantities greater than the blank incubation as a result of Reaction 1, Fig. 34 being active.

5.4 Glutamine Metabolism

5.4.1 The Synthesis of Glutamine

The inclusion of methionine sulfoximine, a non-competitive inhibitor of glutamine synthetase (Meister 1969) in incubations with rumen papillae, reduced the release of glutamate plus glutamine, 19% and 8% with and without added NH_3-N respectively (Fig. 20). Methionine sulfoximine had no inhibitory influence upon the recovery of alanine or glycine with glutamate plus pyruvate (Fig. 21) or glyoxylate (Fig. 22) respectively in incubation media. The reduced glutamate plus glutamine release with added methionine sulfoximine (Fig. 20) was within the standard error of the methods of analysis. It was not possible to determine whether glutamine did constitute a portion of the total glutamate plus glutamine released by rumen papillae. Chalupa et al. (1970) did not report the activity of glutamine synthestase in sheep rumen mucosa, but stated that it was very low; Salem et al (1973a) found an active glutamine synthetase in the rumen mucosa of cattle. In the present study, evidence of glutamine synthetase activity was inconclusive without direct analysis for this enzyme reaction.

5.4.2 The Disappearance of Glutamine from Incubation Media

It was estimated (Appendix C) that 26% of the glutamine added to incubation media (Series II incubations, Figs. 11, 12 and 13) was non-enzymatically cyclized to pyrrolidone carboxylic acid (Reaction 1, Fig. 35) before amino acids were isolated for GLC analysis. Glutamate plus glutamine present at the end of the incubation period could account for no more than 3% of the added glutamine (Table 11); these glutamate plus glutamate recoveries (Figs. 11, 12 and 13) were less than the blank incubation (Fig. 10). It would appear that there was extensive metabolism of glutamine by rumen papillae.

Addition of glutamine to incubation media did not enhance the recovery of glycine from glyoxylate (Fig. 11), or alanine from pyruvate (Fig. 12) or propionate (Fig. 13). This indicated that glutamine was not utilized as an amino donor (Reaction 2, Fig. 35), and that it was probably not converted to glutamate, because when glutamate was added to incubations under similar conditions (Figs. 11, 12 and 13) there were marked amino acid responses (Reaction 5, Fig. 35).

Since the nitrogen of glutamine could not be accounted for in glutamate plus glutamine, nor in amino acids synthesized from carbon sources (Figs. 11, 12 and 13), it was concluded that glutamine was catabolized to a compound for which no analysis was undertaken in the present study. Hoshino

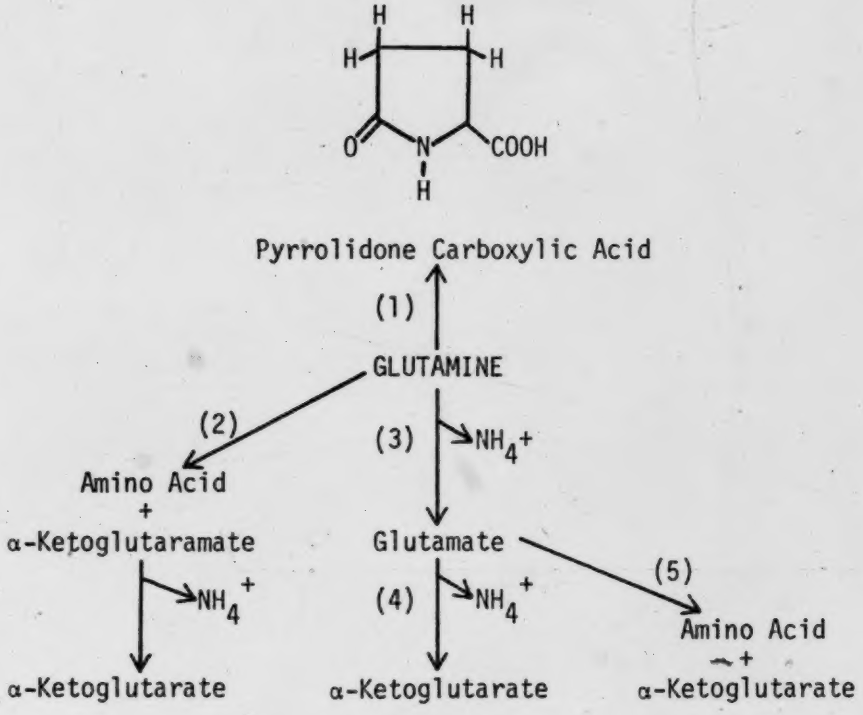

Pyrrolidone Carboxylic Acid
(1)
GLUTAMINE
(2)
(3)
NH_4^+
Amino Acid
+
α-Ketoglutaramate
Glutamate
(5)
NH_4^+
(4)
NH_4^+
Amino Acid
+
α-Ketoglutarate
α-Ketoglutarate
α-Ketoglutarate
COOH

Fig.35. Reactions that may account for the disappearance of glutamine from incubations of glutamine with rumen papillae. (Gilbert et al. 1949; Sallach and Fahien 1969)

Reaction
(1) Cyclization of glutamine to pyrrolidone carboxylic acid
(2) Glutamine-keto-acid aminotransferase (EC 2.6.1.15)
(3) Glutaminase (EC 3.5.1.2)
(4) Glutamate dehydrogenase (EC 1.4.1.2)
(5) Glutamate-keto-acid aminotransferase

et al. (1966) reported that homogenates of rumen mucose were able to hydrolyze glutamine to NH_3-N. An arrangement of glutaminase (Reaction 3, Fig. 35) and glutamate dehydrogenase (Reaction 4, Fig. 35), both mitochondrial enzymes (Knox and Greengard 1965; Sallach and Fahien 1969), where glutamine is shunted directly to produce α-ketoglutarate and NH_3-N might account for release of NH_3-N from glutamine. Mitochondrial membranes contain permeases specific for α-ketoglutarate or glutamate (Williamson 1976). The permeases of mitochondrial membranes may control the exchange of α-ketoglutarate and glutamate between the mitochondrial matrix and cell cytoplasm, favouring the exit of α-ketoglutarate, and limiting the exit of glutamate from mitochondria. It is considered likely that glutamine nitrogen is converted to NH_3-N rather than serving as an amino donor in rumen papillae.

5.5 Glycine-Serine Interactions

Glycine release from rumen papillae was augmented by increasing the concentration of serine (0.1 to 2 mM) in incubation media (Table 13, Fig. 18). Glycine (0.25 to 4 mM) incubated with rumen papillae did not appear to influence serine release (Fig. 19). Upon the addition of 1 mM formaldehyde or 1 mM formate to a 1 mM glycine treatment (Fig. 26), there was an increased serine release by rumen papillae. The interconversion of glycine and serine by rumen papillae may be catalyzed by serine hydroxymethyltransferase (EC 2.1.2.1).

5.6 Arginine Metabolism

Investigations of ornithine cycle enzymes in rumen muscosa of cattle have indicated that arginase (Martincic and Krvavica 1964) is tenfold more active than ornithine carbamoyltransferase (Krvavica et al. 1964) in this tissue. During preparation of amino acid esters (Appendix B) in the present study, citrulline and ornithine appeared to form the same ester. Therefore ornithine release could not be distinguished from citrulline release by a direct analysis of incubation media. One may anticipate that a higher activity of arginase as compared to ornithine carbamoyltransferase would have favoured the formation of ornithine rather than citrulline when rumen papillae were incubated with arginine.

Incubation of rumen papillae with 1 mM arginine resulted in the release of ornithine and indicated the presence of arginase and urea production. Hecker and Nolan (1971) reported no arterio-venous difference for urea concentration across the rumen of sheep,suggesting no net transfer of plasma urea from plasma to rumen fluid across rumen mucosa. Therefore, a flux of nitrogen as blood urea nitrogen demonstrated by Havassy et al. (1974) may not account for the total input of nitrogen as urea into rumen fluid. It may be possible that arginase associated with rumen papillae could catalyze the cleavage of arginine from blood and be responsible for atleast some of the release of nitrogen as urea into the rumen.

5.7 The Influence of Volatile Fatty Acids Upon Amino Acid Metabolism by Rumen Papillae

The volatile fatty acids acetic, propionic and butyric are produced in large quantities as a result of microbial fermentation in the rumen (Hungate 1966) and thus are available in large quantities to rumen papillae. The conversion of propionate to alanine and serine by rumen papillae has already been discussed. Acetate and butyrate (Fig. 27) appear not to influence the release of amino acids by rumen papillae.

5.8 Oxalate and Malonate - Metabolic Inhibitors

Malonate is an effective competitive inhibitor of succinate dehydrogenase (EC 1.3.99.1) (Webb 1966). Webb (1966) stated that inhibition of succinate dehydrogenase results in the accumulation of intermediates of the tricarboxylic acid cycle; succinate accumulated when rumen epithelium was incubated with 0.01 or 0.02 mM malonate plus 40 mM propionate (Pennington and Sutherland 1956b). An increased availability of intermediates of metabolism could lead to the synthesis of amino acids in the presence of a source of nitrogen (NH_3-N or amino acids as amino donors). In the present study, malonate (Fig. 25) had little influence upon the release of amino acids by rumen papillae.

Glyoxylate serves as a source of oxalate in liver tissue; oxalate is not converted to glyoxylate (Weinhouse and Friedman 1951). Although oxalate was added to incubation media as a potential source of glyoxylate in rumen papillae (Fig. 15), oxalate did not selectively increase the release of glycine, suggesting that oxalate was not readily converted to glyoxylate. Oxalate (1 mM and 10 mM, Figs. 25 and 15 respectively) consistently increased the release of glycine, serine, aspartate plus arparagine and glutamate plus glutamine. No increased alanine release was recorded with 10 mM oxalate (Fig. 15) as compared to 1 mM oxalate (Fig. 25) in the incubation media.

The increased release of amino acids by rumen papillae incubated with oxalate (Figs. 15 and 24) was difficult to explain. An inhibition of succinate dehydrogenase in rumen wall by oxalate was reported by James (1968); however,oxalate is a less effective inhibitor of succinate dehydrogenase than malonate (Webb 1966). Malonate had little influence upon the release of amino acids by rumen papillae (Fig. 25). Oxalate precipitates in body tissues as the calcium salt (James et al.1971), and this,in combination with an absence of calcium from incubation media containing oxalate, might have altered the chemical status within the cells of rumen papillae. A change in cell permeability to amino acids was unlikely since the calculated free amino acid concentration of rumen papillae could not account for the release of amino acids by rumen papillae incubated with oxalate. An enhanced proteolysis, with an increased supply of endogenous substrates may explain the effect of oxalate upon the amino acid metabolism of rumen papillae. It was difficult to determine if amino acids other than those reported were released in larger quantities by rumen papillae since the concentrations of these amino acids was very low in incubation media.

5.9 In Vivo Approximations

Important aspects of the in vitro data on the metabolism of rumen papillae were the synthesis of the amino acids alanine, glycine, serine, aspartate (plus asparagine) and glutamate, and the conversion of arginine to ornithine indicating urea production. It was not possible to determine unequivocally whether glutamine was synthesized by rumen papillae. The finding by Hoshino et al.(1966) that glutamine was catabolized to NH_3-N by homogenates of rumen mucosa may explain the low recovery of added glutamine nitrogen as the nitrogen of glutamate plus glutamine or of amino acids after 3 h of incubation. It would be difficult to estimate in vivo nitrogen fluxes in rumen papillae as a result of amino acid metabolism from in vitro data. At best, pathways of nitrogen (rumen fluid NH_3-N) fixation and transformations are indicated in this study.

5.9.1 The Fixation of Rumen Fluid Ammonia Nitrogen by Rumen Papillae

The synthesis of amino acids by rumen papillae, which most likely entails the metabolism of ruminal epithelium, could constitute a means for the fixation of rumen fluid NH_3-N (Fig. 36). In order for a synthesis of amino acids by rumen papillae to occur, nitrogen sources such as rumen fluid NH_3-N and glutamate, and carbon sources such as propionate and glyoxylate must be made available to the tissues of rumen papillae.

Plasma glutamate (Fig. 36) may be used by rumen papillae as a source of amino groups in addition to glutamate synthesized in the tissue. Synthesis of glutamate in the liver using NH_3-N absorbed from the rumen, followed by transport of this glutamate to rumen papillae, and its use as an amino donor, may be an alternate pathway for the ultimate fixation of NH_3-N into amino acids by rumen papillae. Virtually all NH_3-N absorbed by the splanchnic bed, of which the rumen mucosal tissue is a constituent, in sheep fed a high nitrogen diet was reported to be detoxified in the liver and therefore did not reach the peripheral circulation (Wolff et al. 1972). From the data reported by Nolan and Leng (1972), approximately 25% of intraruminally injected $[^{15}N]$-NH_4Cl was retained in a body pool of nitrogen with a slow turnover rate. Plasma glutamate may be a component of this as yet undefined pool of nitrogen which may in fact be a pool of nitrogen that could supply amino groups for the synthesis of amino acids by rumen papillae.

Propionate absorbed from rumen fluid would be expected to be the primary source of carbon for the synthesis of alanine, serine, aspartate (plus asparagine) and glutamate. Organic acids such as pyruvate and

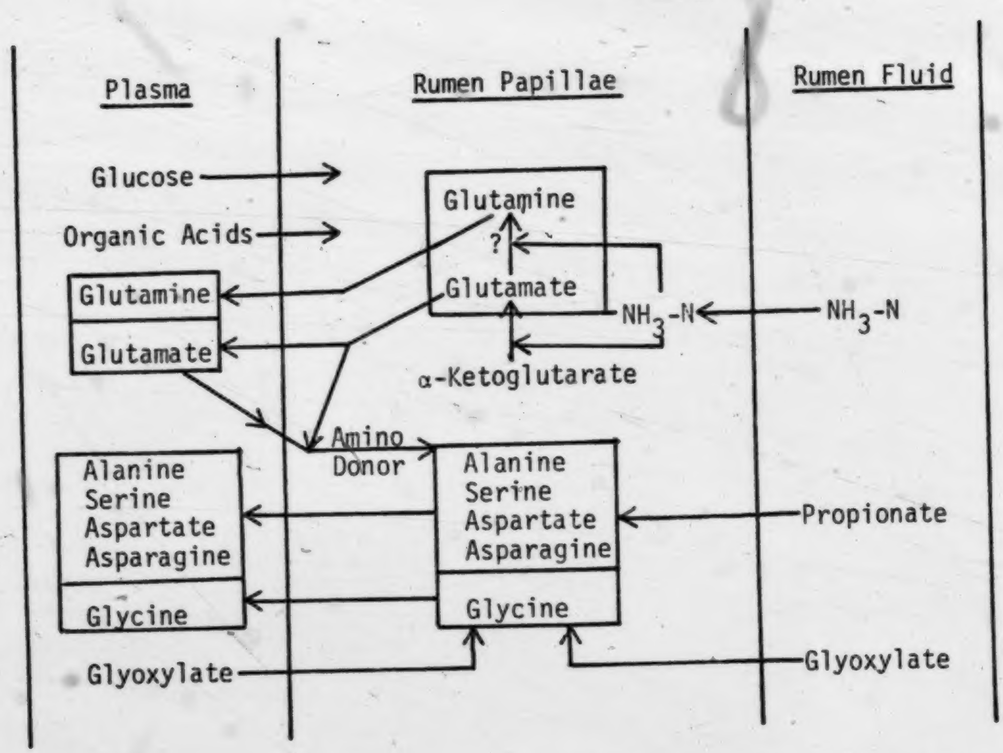

Plasma
Rumen Papillae
Rumen Fluid
Glucose
Organic Acids
Glutamine
?
Glutamate
Glutamine
Glutamate
NH_3-N
NH_3-N
α-Ketoglutarate
Amino Donor
Alanine
Serine
Aspartate
Asparagine
Glycine
Alanine
Serine
Aspartate
Asparagine
Glycine
Propionate
Glyoxylate
Glyoxylate

Fig.36. The possible pathways of fixation of rumen fluid ammonia nitrogen into amino acids by rumen papillae.

and tricarboxylic acid cycle intermediates are present in liver tissue and plasma (Prior et al. 1972) but at very low concentrations. Acetate, which is present in liver tissue and plasma in high concentrations in relation to other organic acids (Prior et al. 1972), did not influence the release of amino acids by rumen papillae.

A possible source of glyoxylate in rumen tissue may have entailed its synthesis from oxalate by bacteria (Kornberg 1966). Plant material can be a source of oxalate (Long 1961). However Talapartra et al. (1948) reported that the major product of oxalate degradation by rumen bacteria appeared to be carbon dioxide.

The lack of an increased glycine release by rumen papillae with hydroxyproline (Fig.24), or the two-carbon compounds glycolaldehyde, glyoxal or glycollate (Figs. 14 and 15) does not, of course, preclude the converion of these compounds to glyoxylate in tissues other than rumen mucosa. Transport of this glyoxylate via the vascular system to rumen papillae might then constitute a source of two-carbon compounds for the synthesis of glycine by rumen papillae (Fig. 36).

In ruminants (Wolff et al. 1972; Cross et al. 1975) glycine is present in plasma at a concentration higher than in monogastrics such as rats (Yamamoto et al. 1974). The concentration of plasma glycine increases twofold when cattle (Brown et al. 1961) or sheep (Cross et al. 1975) are fasted. There is a production of glycine by rumen papillae, as reported herein, in quantities about equal to alanine. In comparison to alanine, glycine is rather ineffective as a glucogenic precursor; glycine and alanine contribute 0.9% and 5.5% respectively of the carbon of glucose turnover in fed sheep (Wolff and Bergman 1972b). Glycine synthesized by rumen papillae may serve an as yet unknown function in ruminants.

5.9.2 Sources of Ammonia Nitrogen to the Rumen

The amino acids, arginine and glutamine, as plasma constituents, are pools of nitrogen that may contribute nitrogen to the rumen (Fig. 37). Arginine was converted by rumen papillae to ornithine in vitro indicating urea production. In vivo, the urea produced in the rumen mucosa would, upon entering the rumen, be degraded to NH_3-N through the action of bacterial urease. When rumen papillae were incubated in the presence of glutamine, the nitrogen of glutamine was neither recovered as the nitrogen of glutamate plus glutamine nor as the nitrogen of the other amino acids. The production of NH_3-N from glutamine may result in glutamine serving the role of a carrier of nitrogen to the rumen microbes.

Bergman et al. (1974a) implicated the plasma constituents, arginine and citrulline as carriers of nitrogen between the liver and kidneys of sheep (Fig. 38). In their studies of amino acids metabolism, an uptake of citrulline by the kidney was nearly balanced by a net output of arginine for fed or fasted sheep. As a result of the present study, a compartment representing rumen papillae was added to the scheme as illustrated in Fig. 38. This could allow an interaction of arginine uptake from plasma and ornithine output to plasma by rumen papillae with the liver and kidney metabolism of arginine and citrulline as suggested by Bergman et al. (1974a). Urea produced in the liver and transported to the rumen would be supplemented by urea produced from arginine by rumen mucosal tissue. Arginine may then be utilized in the transport of nitrogen to the rumen as a result of urea production in the rumen wall.

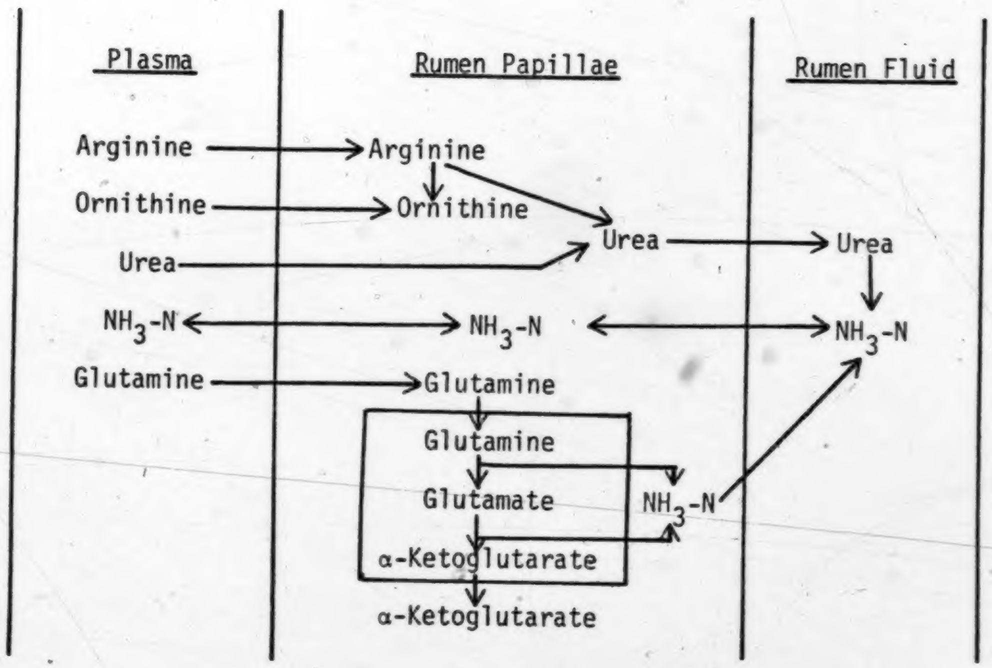

Plasma
Rumen Papillae
Rumen Fluid
Arginine
Arginine
Ornithine
Ornithine
Urea
Urea
Urea
NH_3-N
NH_3-N
NH_3-N
Glutamine
Glutamine
Glutamine
Glutamate
NH_3-N
α-Ketoglutarate
α-Ketoglutarate

Fig.37. Eventual production of ammonia nitrogen in the rumen from plasma arginine and glutamine.

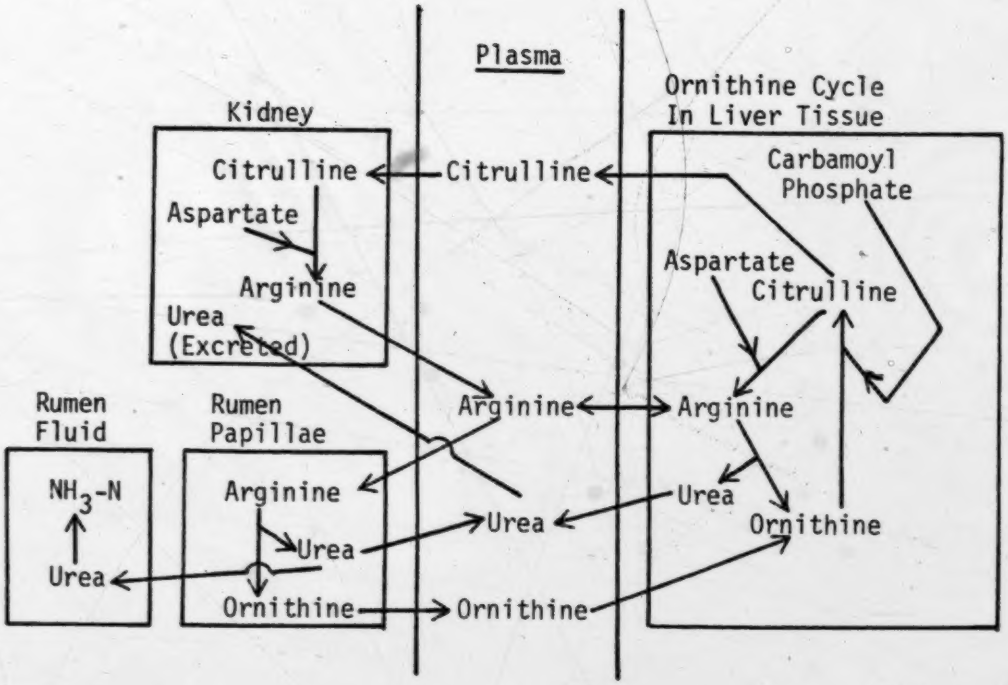

Plasma
Kidney
Citrulline
Aspartate
Arginine
Urea
(Excreted)
Citrulline
Ornithine Cycle
In Liver Tissue
Carbamoyl
Phosphate
Aspartate
Citrulline
Arginine
Arginine
Urea
Ornithine
Rumen
Fluid
NH_3-N
Urea
Rumen
Papillae
Arginine
Urea
Ornithine
Urea
Ornithine

Fig.38. Possible interrelationships of the ornithine cycle amino acids arginine, citrulline and ornithine as carriers of nitrogen in the plasma of ruminants between the kidney, liver and rumen papillae. (Interrelationship between liver and kidney from Bergman et al.1974a; metabolism by rumen papillae, results of the present study)

5.9.3 Rumen Papillae as a Compartment Contributing to the Interorganal Transport of Amino Acids in Ruminants

The nitrogen transformations that may occur in rumen papillae as a result of amino acid metabolism therein have to this point been considered without regard to control of the quantities of amino acids metabolized. In vitro, the synthesis of amino acids by rumen papillae was influenced by the concentration of added carbon substrates (glyoxylate, Table 13, Fig. 16; propionate, Fig. 28) rather than by the concentration of added NH_3-N (Table 13). It would appear that the determining factor of amino acid synthesis by rumen papillae in vivo could be the concentration of carbon sources such as propionate absorbed from rumen fluid. The concentrations of arginine and glutamine in plasma, and possibly of NH_3-N in rumen fluid, may influence the quantity of NH_3-N released from urea produced by the action of arginase upon arginine, or the quantity of NH_3-N released from glutamine.

Although there have been quantitative in vivo tracer studies of the movements of NH_3-N out of the rumen into the rumen wall, and into the rumen from the wall (Mathison and Milligan 1971; Nolan and Leng 1972; Nolan et al 1976), the proportions of these movements that may entail amino acid metabolism in the rumen papillae are unknown. In the present in vitro studies alanine, glycine and glutamate (plus glutamine) were quantitatively the major amino acids produced by rumen papillae; these could be products of rumen wall utilization of NH_3-N in vivo. On the other hand, it was found that rumen papillae readily metabolized arginine and glutamine; these conversions may play a role in nitrogen transport to the rumen in vivo.

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APPENDIX A

PREPARATION OF INCUBATION MEDIA FOR AMINO ACID ANALYSIS

Compounds such as organic acids, which themselves form esters, carbohydrates and ionic components interfere with the gas-liquid chromatographic analysis of amino acids (Gehrke et al.1968; Gehrke and Leimer 1970). A short isolation procedure, using a strong cation exchange resin, was undertaken to remove interfering compounds before amino acid derivatives were prepared.

Amino acids at pH 2.2 to 2.5 are strongly bound to a sulfonated polystyrene resin in the hydrogen form, while organic acids and plasma proteins remain unbound and can be washed through the resin with water (Harris et al.1961). Ammonium hydroxide was used to elute selectively amino acids off the ion exchange resin.

A1. Charging of the Resin

The cation exchange resin was converted to the acid (H^+) form according to the method of Pellizzari et al.(1971). The resin, AG50W-X8 (H^+) (200-400 mesh) (Bio-Rad Laboratories Ltd.; Richmond, Calif. U.S.A.), a sulfonated polystyrene was suspended in distilled deionized water with stirring and the slurry was adjusted to about pH 12 with 20 N NaOH. Water was deionized by passing distilled water through a Barnstead demineralizer cartridge (Sargent-Welch Scientific Company; Toronto, Ont., Canada) containing multibed cation and anion exchange resins. After stirring for 20 minutes, the resin was washed with distilled, deionized water until the supernatant was about pH 7.0, and then converted to the acid form in a slurry of 6N HCl.

A second wash of 6N HCl was used to complete the conversion of the resin to the acid form. Enough 6N HCl was added in each wash to suspend the resin in a slurry while stirring. The charged resin was washed with distilled, deionized water until the supernatant was about pH 4.0 and was stored in distilled deionized water at 0 C.

The resin bed volume used for each incubation was 1.0 ml and represented 1.2 meq of exchange capacity (determined according to Harris et al. 1961). Ion exchange columns were similar in construction to those used by Harris et al (1961).

A2. Isolation of Amino Acid for Analysis

Incubation media, containing an internal standard and adjusted to pH 2.2 to 2.5 with a 0.1 volume of 8N acetic acid (Harris et al. 1961), were added to each column of prepared ion exchange resin and washed into the column with 0.5 ml 0.1N acetic acid. The resin was washed with 10 ml of distilled deionized water. Amino acids were eluted with 10 ml of 1N NH_4OH followed with a 5 ml distilled deionized water wash into a 20 ml test tube held in ice. The fractions were mixed thoroughly and divided into two 13 x 100 mm culture tubes with teflon lined screw caps (Canlab; Edmonton, Alta., Canada), frozen immediately at -30 C and lyophilized. The lyophilized residues were stored at -30 C until amino acid esters were prepared. The culture tube with the lyophilized residues served as the reaction tube for amino acid ester formation.

A3. Recovery of Amino Acids

The recovery of amino acids from the ion exchange procedure depended upon the normality of the eluting base (NH_4OH) and upon the ability of the eluting base to displace amino acids from the ion exchange resin. The recovery of all amino acids from a standard solution representing a protein hydrolysate (Appendix B), except lysine and arginine was 90% or better. An 80% recovery of each of lysine and arginine was achieved.

The use of NH_4OH as an eluting agent at a concentration 2N or greater resulted in poor recoveries of the basic amino acids lysine and arginine. Since the pKa's of NH_4OH and arginine were not very different, the ability of NH_4OH in dilute solutions to displace arginine from the cation exchange resin (Davies 1949) was reduced. The recovery of all amino acids from the resin was maximized with the ion exchange procedure presented.

APPENDIX B

THE GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF AMINO ACIDS

Amino acids were analyzed as the isobutyl-N(O)-heptafluorobutyryl esters using a modification of the procedure of MacKenzie and Tenaschuk (1974).

B1 Instrumentation

A Bendix 2500 gas chromatograph (Bendix Corporation, Process Instruments Division; Ronceverte, West Virginia, U.S.A.) equipped with a four column oven, four flame ionization detectors and dual differential electrometers was used. Peak integration values were obtained with an Autolab Minigrator (Spectra Physics, Technical Marketing Associates, Mississuaga, Ont., Canada) and the peaks were reproduced on a Fisher Recordall Series 5000 (Fischer Scientific Company; Edmonton, Alberta, Canada).

B2 Preparation of Columns and Packing Material

A pair of glass columns, 3.7 m long and 5 mm OD were prepared to conform to an on column injection of samples. A column packing of 3.5% (wt/wt) OV-1 on Gas-Chrom Q (80-100 mesh) (both OV-1 and Gas-Chrom Q from Applied Science Laboratories Inc.; State College, Pa., U.S.A.) was prepared. The OV-1 was dissolved in redistilled chloroform overnight and added to the solid phase in a round bottom flask; excess chloroform was added to the solid phase such that the solid phase was completely immersed in the chloroform solution. The solvent was slowly removed under vacuum on a rotary evaporator. Glass wool was used to plug the detector ends of the columns and the packing material was added to each column. The columns were conditioned, and maintained when not in use, at an oven temperature of 250 C and a nitrogen flow of 30 ml/min.

B3 Instrumental Conditions

Inlet temperature		250 C
Detector temperature		290 C
Temperature program		100 C for 5 min, 8 deg/min. to 230 C, 4 deg/min 230 to 260 C
Gas flows	Nitrogen	30 ml/min at 40 lb/in^2
	Air	360 ml/min at 20 lb/in^2
	Hydrogen	40 ml/min at 2[illegible] lb/in^2

B4 Reagents

Isobutanol and methylene chloride, both obtained from Fisher Scientific Company, were redistilled to a constant boiling point. Heptafluorobutyric anhydride was purchased from the Pierce Chemical Company (Rockford, Ill., U.S.A.).

B5 Preparation of Isobutanol-HCl

Hydrogen chloride gas (Matheson of Canada Limited; Whitby, Ont., Canada) was bubbled through isobutanol until the alcohol was 3.0 N HCl. The normality was determined by titration with 0.1 N NaOH.

B6 Amino Acid Standards

A mixture of amino acids representing a protein hydrolysate (hydrolysate standard) containing the amino acids L-alanine, glycine, L-valine, L-threonine, L-serine, L-leucine, L-isoleucine, L-proline, L-methionine, L-aspartic acid, L-phenylalanine, L-glutamic acid, L-lysine·HCl, L-tyrosine, L-arginine·HCl and L-histidine·HCl at 2.5 μmoles each per ml aqueous 0.1 N HCl, and L-cystine at 1.2 μmoles per ml aqueous 0.1 N HCl was prepared. These amino acids plus the amino acids L-asparagine·H_2O, L-glutamine, L-cysteine, hydroxy-L-proline and L-tryptophan were obtained in Kit Number LAA-21 from Sigma Chemical Company (St. Louis, Mo., U.S.A.). Additional amino acids purchased were L-ornithine·HCl and L-citrulline from Sigma Chemical Company, cycloleucine (1-aminocyclopentanecarboxylic acid) from Aldrich Chemical Company (Milwaukee, Wis., U.S.A.) and D,L-O-phosphoserine and L-norleucine from Calbiochem (Los Angeles, Calif., U.S.A.). Cycloleucine was the internal standard for all preparations of Series I to Series VI.

B7 Amino Acid Preparation for Chromatography

All reactions were carried out in 13 x 100 mm culture tubes with teflon lined screw caps (Canlab; Edmonton, Alta., Canada). Two water baths (Precision Scientific Company; Chicago, Ill., U.S.A.) containing a bath medium of refined rapeseed oil were adjusted to either 100 ± 2 C or 150 ± 3 C.

For a standard mixture of amino acids in aqueous 0.1 N HCl, without ion exchange preparation, the procedure for ester formation was started at Part A (below). Lyophilized preparations, after ion exchange preparation

(Appendix A) of a standard mixture of amino acids or of incubation media were started at Part B (below).

A. Removal of Water. A 100 µl sample of amino acid standard and 100 µl of internal standard (2.5 µmoles per ml aqueous 0.1 N HCl) were added to the reaction tube and dried in a vigorous stream of nitrogen at room temperature.

B. Isobutylation. Two hundred microlitres of isobutanol - 3.0 N HCl was added to the dry amino acid residues. The tube was gently flushed with nitrogen, capped tightly, and mixed for 15 seconds on a Vortex mixer (full speed). After esterification in an oil bath at 100 C for 30 min, the reaction vessel was removed from the bath and the isobutylation reagent was evaporated to dryness under a stream of nitrogen.

C. Acylation. Two hundred microlitres of heptafluorobutyric anhydride (HFBA) and 100 µl of methylene chloride were added to the dry isobutyl esters. The tube was gently flushed with nitrogen and capped tightly. The mixture was allowed to react in an oil bath at 150 C for 10 min and was then cooled to room temperature. The acylation reagent and solvent were evaporated under a gentle stream of nitrogen.

Reaction tubes with dry amino acid esters were flushed with nitrogen, tightly capped and stored temporarily in an ice bath, or in a freezer at -30 C until the esters were chromatographed. Before injection, the derivatives were dissolved in 50 µl of methylene chloride. In all cases one µl of HFBA was coinjected with the amino acid esters.

B8 Amino Acid Analyses

With the exception of histidine, amino acids present in a prepared standard mixture representing a protein hydrolysate produced a similar chromatogram (Appendix Fig. B1) to that reported by MacKenzie and Tenaschuk (1974). Coinjections of HFBA resulted in histidine being chromatographed between phenylalanine and glutamate, while coinjection with acetic anhydride (MacKenzie and Tenaschuk 1974) resulted in histidine being chromatographed after arginine (Appendix Fig. B1).

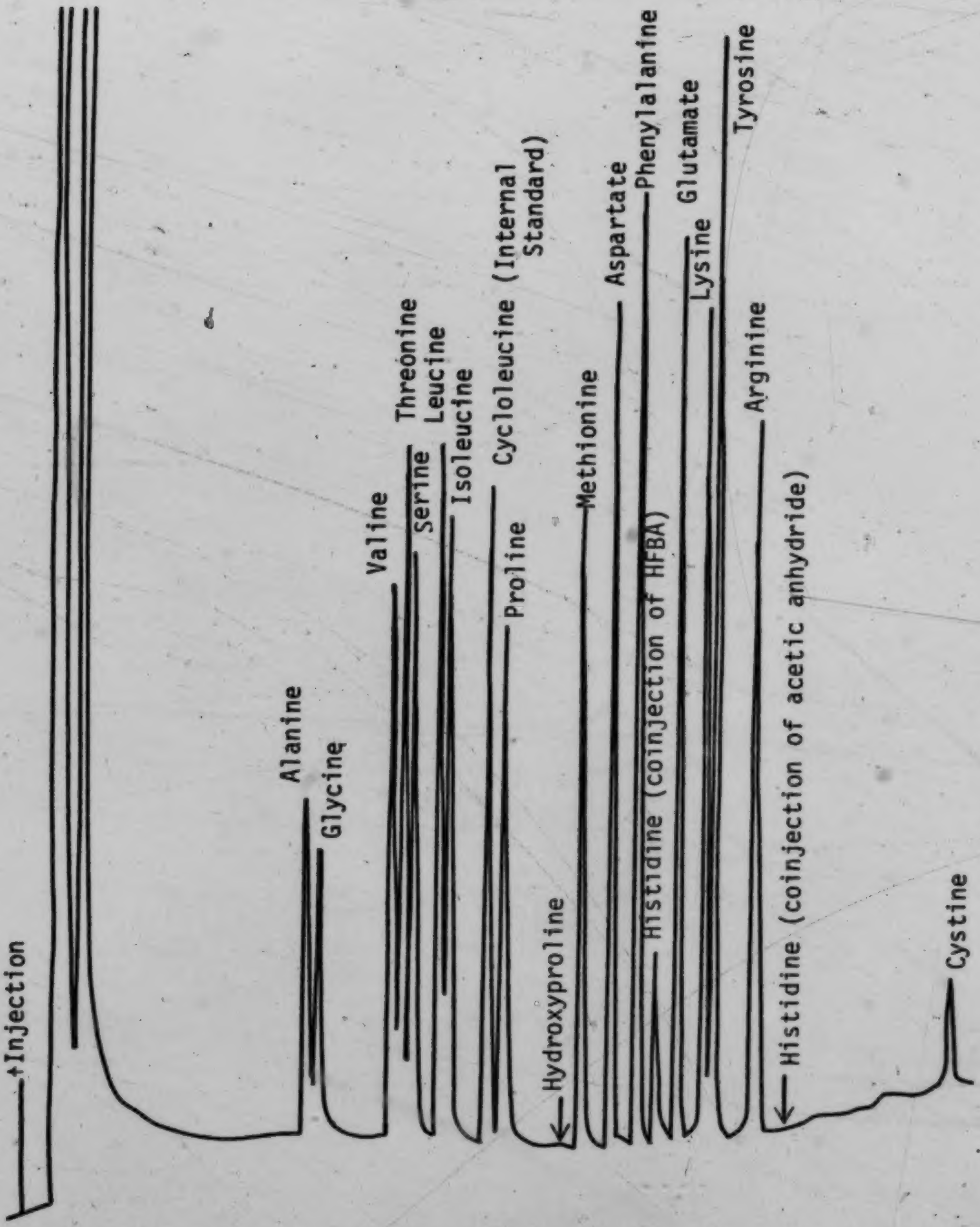
A chromatogram of amino acids from an incubation without added nitrogen or carbon substrates is presented in Appendix Fig. B2. The amino acids of interest in this preparation are alanine, glycine, serine, aspartate plus asparagine, glutamate plus glutamine, ornithine and arginine. Hydroxyproline would be chromatographed at the position marked in Appendix Fig. B1; this position relative to the other amino acids is in agreement with MacKenzie and Tenaschuk (1975).

B9 Limitations of Amino Acid Analysis

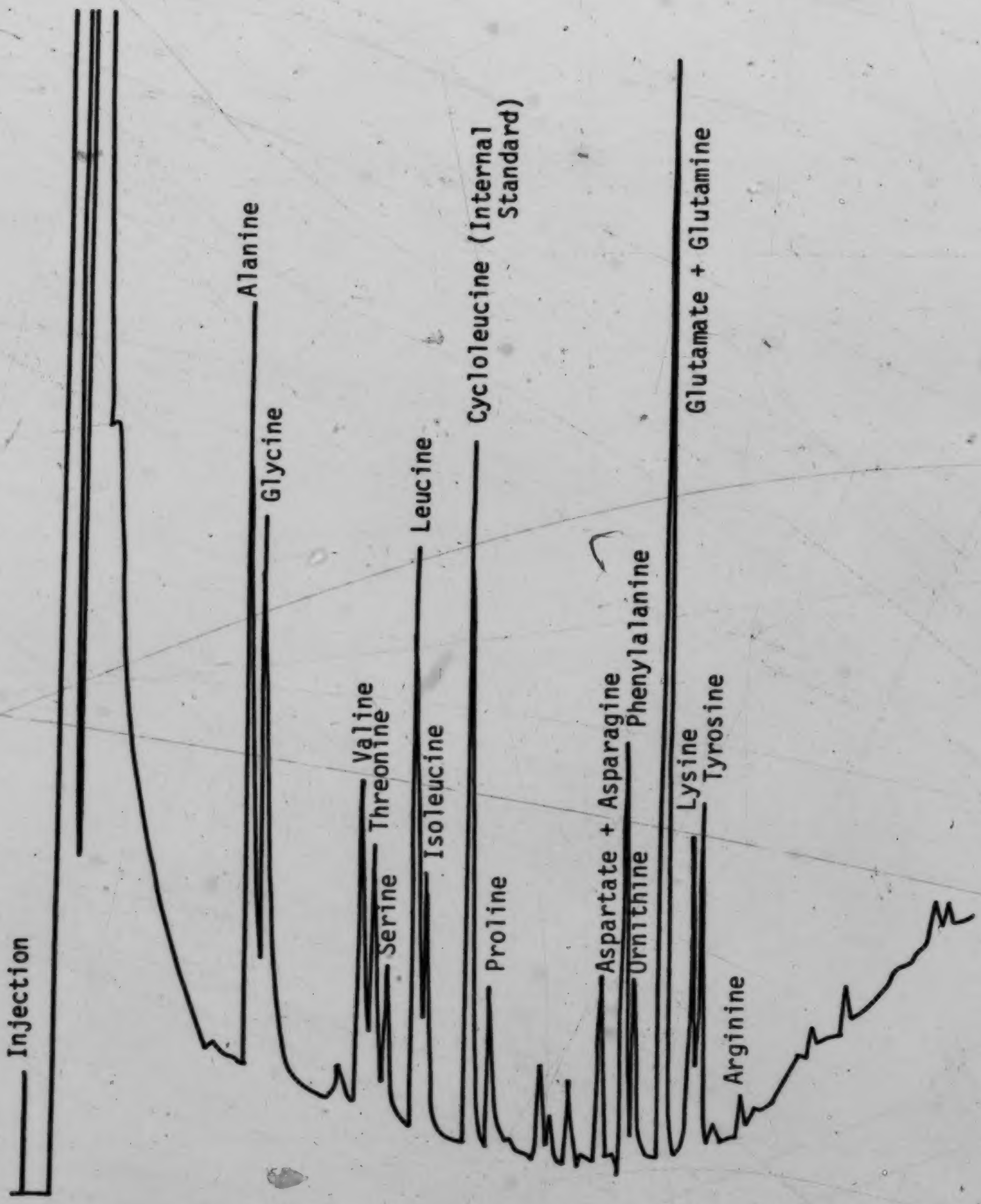
No difficulties were encountered with the analysis of alanine, glycine or hydroxyproline.

The amides, glutamine and asparagine were chromatographed as the dicarboxylic acids, glutamate and aspartate respectively. With a standard mixture of glutamate, glutamine, aspartate and asparagine, two and not four peaks were found in the chromatograms. Acid calalysis, in isobutanol—HCl at 100 C, would have caused the release of the amide group with the respective dicarboxylic acid being formed (Morrison and Boyd 1972).

Phosphoserine and serine had the same retention characteristics. It appeared that the phosphate group was cleaved from phosphoserine to form serine quantitatively under the conditions of amino acid ester formation.



Appendix Fig.B1. A typical chromatogram of the isobutyl-N(O)-heptafluorobutyryl esters of amino acids that may be present in a protein hydrolysate with cycloleucine as the internal standard. The positions of hydroxyproline,and histidine with a coinjection of acetic anhydride, relative to the other amino acids, were noted. Injection of 5 nmoles of each amino acid except cystine at 2.5 nmoles, coinjected with heptafluorobutyric anhydride (HFBA). Column:3.5 % OV-1 on Gas Chrom Q (80 to 100 mesh), 3.7 m X 5 mm OD glass. Conditions: 100 C for 5 min, 8 deg/min to 230 C, 4 deg/min 230 to 260 C.



Appendix Fig.B2. A chromatogram representing the gas-liquid chromatographic analysis of amino acids from an incubation of rumen papillae with no added nitrogen or carbon sources. Conditions noted in Appendix Fig.B1.

Ornithine was quantitatively chromatographed as a single peak. During derivative formation citrulline breaks down and yields ornithine; this conversion of citrulline to ornithine was incomplete.

The complete conversion of glutamine to glutamate and asparagine to aspartate, and the incomplete conversion of citrulline to ornithine has recently been reported by Siezen and Mague (1977).

The coinjection of HFBA was necessary to obtain a repeatable recovery of the esters of serine and arginine. It was believed that the HFBA forced the acylation reaction to completion in the injection port at 250 C.

APPENDIX C

THE NON ENZYMATIC CYCLIZATION OF GLUTAMINE TO PYRROLIDONE CARBOXYLIC ACID

Gilbert et al. (1949) demonstrated that the non-enzymatic cyclization of glutamine to pyrrolidone carboxylic acid (PCA; Reaction 1, Fig. 35), is accelerated in the presence of bicarbonate or phosphate. To determine how much of the glutamine added to incubation media (Incubation of Series II, Figs. 11, 12 and 13) is cyclized to PCA, a solution of 1 mM glutamine in Krebs-Ringer Bicarbonate (KRB, adjusted to pH 7.0) was analyzed with and without incubation at 39 C for 3 h.

C1 Behaviour of Pyrrolidone Carboxylic Acid During the Ion Exchange Procedure for Isolation of Amino Acids

Owens et al. (1953) used Dowex 50 in the acid (H^+) form to separate amino acids from the organic acids present in the juice of sugar beets. The PCA present in the sugar beet juice was recovered in the effluent water wash fractions with the organic acids but not in the amino acid fractions which remained bound to the Dowex 50 (H^+) ion exchange resin. Both the Dowex 50 (H^+) used by Owens et al (1953) and the AG 50 W (H^+) used to isolate amino acids from incubation media in this study (Appendix A) are sulfonated polystyrene cation exchange resins. In solution at a pH 2.2 to 2.5 glutamine was bound to the resins, while PCA would have passed through with the first 10 ml wash fraction. Glutamine was recovered from the resin by elution of the column with 1 N NH_4OH.

C2 Analysis for the Disappearance of Glutamine From a Solution in Krebs-Ringer Bicarbonate

Glutamine was isolated and determined quantitatively according to the methods presented in Appendices A and B.

A 4 ml volume 1 mM glutamine in KRB (source of buffer chemicals, Table 3) was incubated for 3 h using the procedure described for rumen papillae in the Materials and Methods section. A 1 ml volume of cycloleucine internal standard was added to 2 ml of glutamine in KRB that had been incubated for 3 h at 39 C; a 2 ml aliquot of glutamine in KRB that had not been incubated, was also prepared. The glutamine in KRB, with added internal standard, was adjusted to pH 2.2 to 2.5 with 8 N acetic acid (Harris et al. 1961) and glutamine was isolated from a 300 µl aliquot of this mixture.

C3 Losses of Glutamine from Krebs-Ringer Bicarbonate

The loss of glutamine in the three days before analysis for glutamine was 20% of that present in a prepared 1 mM solution of glutamine in KRB. This was followed by a further loss of 6% of the 1 mM glutamine in KRB after incubation for 3 h at 39 C. Cyclization of glutamine to PCA might account for the disappearance of 26% of the 1 mM glutamine included with the carbon sources glyoxylate (Fig. 11), pyruvate (Fig. 12) and propionate (Fig. 13) in the incubations of Series II.

Appendix Table 1. Percent dry matter of rumen papillae used for the preparations of Series I to Series VI.

Series	Day	Percent Dry Matter	Standard Error
I	1	17.7	0.2
	2	18.6	0.2
	3	18.3	0.4
II	1	17.1	0.3
	2	17.9	0.2
	3	16.1	0.1
III	1	19.5	0.2
	2	18.2	0.4
	3	18.0	0.3
IV	1	17.9	0.3
	2	18.5	0.3
	3	19.8	0.1
V	1	18.6	0.3
	2	17.5	0.3
	3	16.1	0.3
VI	1	18.0	0.5
	2	17.9	0.3
	3	19.1	0.3
Average (n=18)		18.0	0.2